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Preparation and Characterisation of Gellan Gum and Eudragit E100 Polyelectrolyte Complexes

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Abstract

Over the past few years, polyelectrolytes have been employed in several fields across the world to be very functional. They have been used in a wide array of industries such as pharmaceuticals, tissue- engineering and also cosmetics due to their excellent properties such as their ionic strength, adaptable pH, rate of solubility dissolution and also the viscosity. These polymers are so versatile that they can be tailored to how they need to be used, to do this minimal changes are made to the functional groups which then modify the whole polyelectrolyte and can be designed for a set target. Effective polyelectrolytes can be developed by selecting appropriate polymers which are positively and negatively charged. The aim of this study was to select and prepare polyelectrolyte complexes from oppositely charged polymers gellan gum and Eudragit E100. Furthermore, the ideal complex weight ratio was established after characterising certain factors such as the viscosity, zeta potential and the turbidity. Additionally, the complexes were further characterised using FTIR to confirm complexation, XRD to determine the crystallinity or amorphous properties of the polyelectrolytes, thermal analysis was also carried out using DSC and TGA techniques and surface morphology by SEM. In addition to this, model drugs ibuprofen and propranolol were formulated as part of tablets and also as beads and were then further characterised by dissolution studies. The results collected from the overall data confirmed the anticipated complexes. Overall, the study carried out proved that the complexation reaction between the two polyelectrolytes was effective and provided promising results.

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Chapter 1: Introduction and literature review

Polyelectrolytes

Polyelectrolytes have been a very popular subject for scientific research for the past few decades because of their significance in pharmaceuticals and molecular biology. Polyelectrolytes are polymers that comprise of positively charged groups or negatively charged groups dependent on the pH. However, they only act in this way at a neutral pH. There are various kinds of polyelectrolytes such as synthetic, naturally occurring and naturally modified (Schanze & Shelton, 2009). Furthermore, they are very large in structure which can also be referred to as macromolecules, such polymers can readily dissolve in solvents for example water, this is because water is polar and has numerous covalently bonded groups attached (Budd, 1989). In addition to this, polyelectrolytes are very versatile because the physicochemical properties such as the viscosity, positive/negative charge, solubility rate, pH etc can be transformed and controlled completely (Dhakara & Anajwala, 2010).

The pharmaceutical industry in particular has found countless ways to improve and progress with novel drug delivery systems. An array of methods such as nanoparticle drug delivery has been studied extensively to improve drug delivery. Polyelectrolyte complexation is one of the many methods used in industry as part of formulations such as part of numerous formulations. For example, nanoparticles were found to have useful properties such as managing the control of the delivery but also the number of side effects had also reduced (Tan et al. 2016). A number of other approaches involving complexation have shown to be very promising for formulations such as the use of smart polymers. Examples of smart polymers include Carbopol, Sulfonamide and L-Histidine (Mahajan & Aggarwal, 2011). These polymers are able to react upon a change in their environment for example if the pH was to be changed or the temperature (Srivastava et al. 2016). Smart polymers can also be used as part of complexation and have been shown to be successful because specific polymers are combined according to their properties so they are biocompatible and give optimum results (Lankalapalli & Kolapalli, 2009).

Examples of polyelectrolytes

Table 1- Examples of polyelectrolytes

Polyelectrolyte Category	Polycation	Polyanion
Natural	Lysozyme, Starch, Dextran, Gelatin, Chitosan	Gellan Gum, Sodium Alginate, Gum Karaya, Catappa Gum, Nucleic Acids, Hyaluronic Acid, Carrageenan
Synthetic	Chitosan derivatives, Chitosan-G-polyethylene glycol, N-trimethyl Chitosan	Pectin, Xanthan Gum, N-carboxymethyl Chitosan, Sodium Dextran Sulfate, Carboxymethyl Konjac Glucomannan
Semi-synthetic	Eudragit E100, Eudragit® E PO, Poly (methacryloxyethyl trimethylammonium chloride), Poly (allylamine hydrochloride), Poly (β-amino ester, Poly (vinylbenzyl trialkyl ammonium), Poly(2-vinylpyridine), Poly(sulfone-amine) hydrochlorate, Poly(ethyleneimine), Poly (aminoethyl methacrylate), Poly(2-ethyloxazoline), Poly (diallyldimethyl ammonium chloride)	Eudragit L100, Eudragit S100, Poly (sodium acrylate), dextran sulfate, poly (vinyl sulfate), Poly (acrylic acid)/Carbopol, Poly (sodium styrene sulfonate), Poly (methacrylic acid), Poly (sodium 4-vinylbenzoate), Poly (itaconic acid), Poly (acrylic acid-co-maleic acid), poly (metaphosphoric acid), Poly (vinyloxy-4-butyric acid), Poly (acrylamide-2-methyl-propane sulfonate)

A comprehensive review on polyelectrolyte complexes, 2017

- ### 1.2.1 Cationic Polymers

Cationic polymers are positively charged and have the ability to be used as excipients for formulations. Some examples of cationic polyelectrolytes are Chitosan, Gelatin, Dextran, Cellulose, Eudragit E100. For example, Gelatin is a naturally derived polymer from collagen through two processes, one being an acidic procedure which requires the use of an acidic medium and the other being an alkaline procedure which requires an alkaline medium. This is a hydrolysis reaction via catalysis undergoing thermal denaturation (Mariod & Adam, 2013)

Gelatin has several applications such as being used as an excipient in drug formulations because of its good compatibility properties with other excipients (Samal et. al. 2012).

- **1.2.2 Anionic Polymers**

Anionic polymers are macromolecules negatively charged in nature. Some examples of anionic polyelectrolytes are Gellan Gum, Sodium Alginate, Gum Karaya, Eudragit L100, Carrageenan etc. Such polyelectrolytes have been widely used in drug delivery as tablets, ophthalmic medications, oral solutions etc. For example, Sodium Alginate was utilised in a study as an anionic polyelectrolyte to formulate extended release tablets with a combination of active drugs Theophylline and Metoprolol succinate (Li et. al. 2014).

Polyelectrolyte interactions /complexation reactions

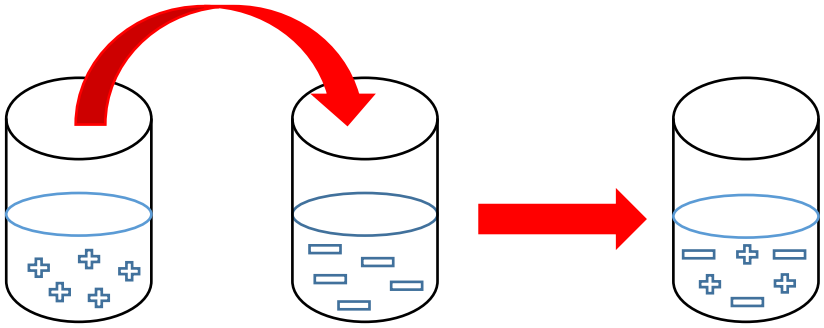

Figure 1- Complexation reaction between gellan and eudragit

Figure 1 shows the basic interaction between Gellan Gum and Eudragit E100, Gellan was poured dropwise into the Eudragit solution to form the final complexes. The complexes obtained were the Gellan gum and Eudragit E100 complexes due to electrostatic bonding.

Researchers in the field of pharmaceuticals agree upon the reaction of polyelectrolytes as entropy driven. The polyelectrolyte complex formation progression takes place very quickly upon addition of the other polymer and in under than 5 seconds. The forces involved in this reaction comprise of strong electrostatic interactions between the two oppositely charged polymers. The overall mechanism of the polyelectrolyte complexes can be seen as an illustration in figure 2 (Dautzenberg et al. 2003).

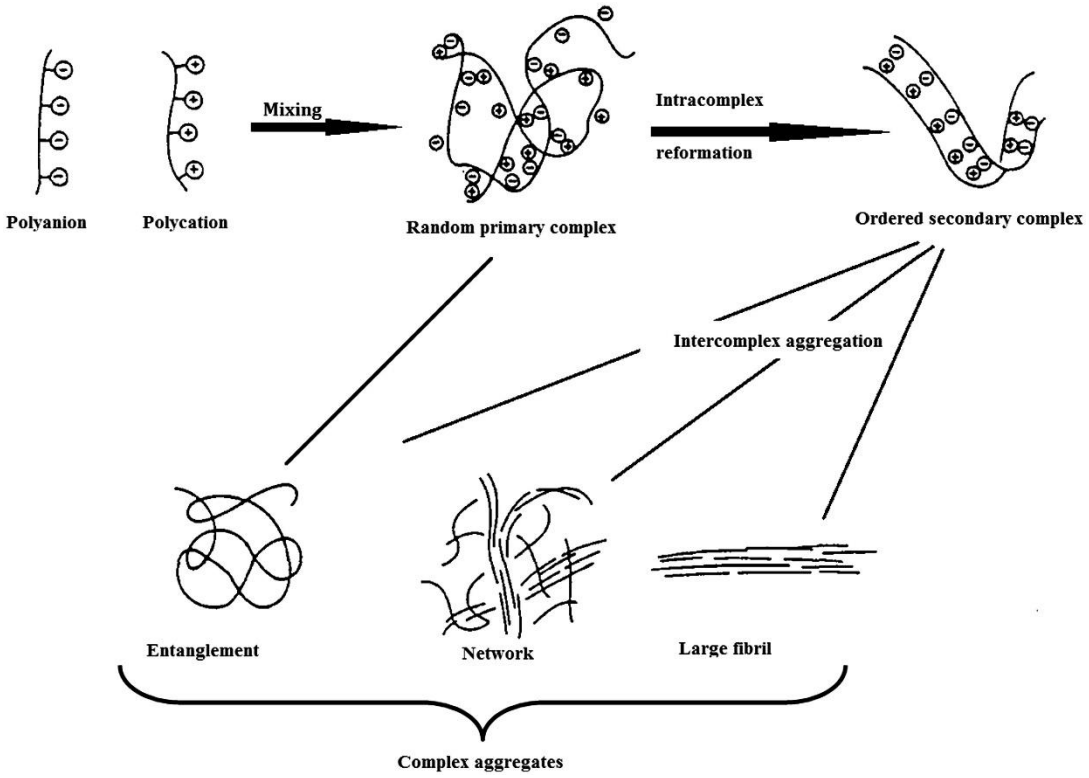

Figure 2- Complexation reaction between polyanion and polycation

(Kulkarni et al. 2016)

1. The initial stage begins when the oppositely charged polyelectrolyte solutions come into contact and an electrostatic charge links them upon agitation, this process happens very quickly and results in the primary complex formation.
2. The next stage of the reaction involves the development of new bonds including Coulomb's reactions, hydrophobic interactions and hydrogen bonding. This is where polymer structures take a new conformation. This process takes around 1-2 hours from the time of stirring and is known as intermediate complex formation.
3. The intermediate complexes now accumulate with the aid of hydrophobic interactions between the polyelectrolytes and the aqueous media. Also, there are some remaining

aggregates remaining which are not soluble in some solvents (Abhijeet et al. 2016) & (Wu & Delair, 2016).

The types of interactions found between polyelectrolyte complexation reactions involve Coulomb's interactions, hydrogen bonding, hydrophobic interactions, electrostatic interactions, Van der Waals forces and also dipole dipole forces. Furthermore, it was found that the core bonding between the cationic and anionic polyelectrolytes was Coulomb's interactions and this was what caused the complexation process to occur (Delair, 2011).

Factors affecting complexation reactions

There are a number of factors that have been shown to effect on the formation and properties of PECs such as the, polymer concentration, ionic charge, mixing ratio, addition order, pH, mixing time, complexation duration for full complexation, molecular weight of polymer, temperature/humidity etc. PECs are oppositely charged biopolymers that can be complexed together to produce a new polymer. Such factors can have a major impact on the final complexes because the factors like the viscosity of the polyelectrolyte can be reduced, the turbidity, zeta potential and solubility of the polymer can also be affected followed by other limitations such as a loss of yield of the final product. Since PECs can behave sensitively to many factors like previously mentioned, controlling the conditions is considered to be very important (Abhijeet et. al. 2016). The turbidity is a measure of how cloudy or clear a sample is, the higher the absorbance of the sample, the more complexation has taken place.

- ### 1.3.1.1 Polyelectrolyte concentration

The polyelectrolyte concentration of a certain polymer is of great significance because of the huge effect it can have on the polyelectrolyte complex this result would only occur if the polymers are in solution. (Machinskya & Vasilevskaya, 2016) described a study of a complexation reaction between a polyanion and a polycation and reported that a higher volume of the polyanion (negative) resulted in a reduction of ionisation in the complex formation. Additionally, the same experiment reiterated that a fixed concentration of the polymer solution is imperative because it showed to improve the swellability. The solubility rate also showed an increasing trend when an active drug was entrapped within the complex.

- **1.3.1.2 Mixing ratio**

The mixing ratio of the polymers has a crucial influence on the overall polyelectrolyte complexation reaction. (Lankalapalli & Kolapalli, 2012) stated that there was a noticeable difference in the final yield obtained when different weight ratios were tested using the polyelectrolytes gum karaya and chitosan. Furthermore, it was found that when polymer solutions of both polyelectrolytes were mixed together, a larger amount of the complexation yield was noticed when a larger amount of chitosan was added. Maximum complexation was attained at a 15:1 ratio (gum karaya: chitosan). A model study carried out by (Son et al. 2006) presented the outcome of how the mixing ratio variation effected the complexed films. Polyelectrolytes chitosan and polyethylene glycol monosuccinate were complexed at different weight ratios but the film formation only occurred at 20:80 and 40:60 volumes in an acidic environment at a pH between 4-5.

- **1.3.1.3 Addition order**

The order of addition, in which the way the polyelectrolytes are mixed together has a major influence on the complexation process. (Yilmaz et al. 2019) described results from an experiment and found that when the polyanion was added to the polycation a stronger binding interaction was observed between the two polyelectrolytes which resulted in better complexation. Likewise, (Yengchao, 2014) presented a study between chitosan and GG and brought forward how the order of addition impacted the complexation. A chitosan film was placed and dipped into GG polymer solution, which resulted in outstanding mechanical properties and showed an increased tensile strength but when the experiment was performed the opposite way where GG films were dipped into chitosan. These advantageous properties had decreased. In addition, another study stated that the addition order of a polyelectrolyte to the other polymer had an effect on the particle size produced from the complexes. A larger particle size was attained by using a higher concentration of alginate than chitosan but a reduced particle size was obtained when a higher concentration of chitosan was used for cationic PECs (Saether et. al. 2008).

- **1.3.1.4 pH**

The pH is also a crucial factor in the formation of PECs, phase changes happen when the pH is altered (Muller et al. 2011). Correspondingly, (Lankalapalli & Kolapalli, 2012) confirmed how an increase pH effects the complexation reaction. The experiment involved gum karaya

and chitosan where the pH was slowly increased to 4.5 from an acidic lower pH. It was seen that the final yield of the complexes had decreased infinitely at this new pH, this was due to agglomeration occurring due to the anionic charge being overcome by the pH being increased. (Moustafine et al. 2005) also reported a study between sodium alginate and Eudragit E100 complexes. The results indicated that there was a decrease in the number of groups being protonated on the Eudragit polymer when the pH was increased.

- **1.3.1.5 Temperature**

Temperature has an integral part during a complexation reaction and must be maintained at a constant rate because of properties such as viscosity, solubility and polyelectrolyte stability. If a variance occurs, then these factors would also have an effect on the final complexes. A study showed that as the temperature was increased, the pH had been affected. The change in pH showed a decreasing pattern due to the protons detaching and dissociating with an increase of thermal energy (Taherkhani et al. 2017).

Applications of polyelectrolytes

- **Pharmaceutical applications**

Polyelectrolytes have a pivotal role in a number of industries but in particular the medicinal and pharmaceutical industries. The reason for this is because these specific polymers have proved to have excellent bioavailability, increased dissolution rate, improved solubility and enhanced bioavailability. Polyelectrolytes have been used widely as part of medical formulations in the form of excipients such as coating layers, matrices and even as binding agents. A benefit of this is that such polymers can be used in a variety of ways for example, a controlled release could be achieved with the use of a coating layer according to how quickly or fast the active drug needs to be released from the overall formulation and absorbed by the patient with the best effect possible. In addition to this, polyelectrolytes have been used as an overall matrix for many drug delivery systems such as oral, buccal and nasal drug forms. Effective results have been obtained because polyelectrolytes behold good adhesion properties resulting in better drug attachment to the site of the target (Vandenberg, et al. 1989).

Other examples of polyelectrolyte pharmaceutical applications include the polymers chitosan and dextran sulfate as nanoparticles, including the active curcumin for the treatment of cancer (Anita et al, 2011). Another active tenofivir is used with the same biopolymers for the treatment of hiv/aids (Polexe et al. 2013). Furthermore, polymers tmc and alginate have also been complexed to be used as a treatment for cancer including the drug curcumin (Martins et al. 2013).

Moreover, polyelectrolytes have been extensively researched upon as drug entrapped complexes in the form of injections and films along with other drug delivery systems. In addition, these particular polymers have the capacity to swell and expand for a set amount of time before it releases the active drug inside. An example of this would be Gellan Gum because it has the ability to swell rapidly upon the addition of water for instance, any active drug entrapped on the inside would go through a lag phase before it can release through the gel layer. A polyelectrolyte complexed matrix or even a coating layer behaves based on the pH it comes into contact with. For instance, a drug delivery system requiring a sustained release profile would be ideal because the external layer would respond differently. It was found that Poly-acrylic polyelectrolytes have been studied extensively as topical ointment creams so a steady rate of release was achieved over time for a longer lasting effect (Dautzenberg et al. 1994).

- **Non-pharmaceutical applications**

Polyelectrolytes have been widely used as part of non- pharmaceutical related uses. For example, Gellan Gum, also a hydrocolloid is a very broadly used polyelectrolyte particularly in the food industry. This polymer has been used as a fruit juice gel which is something very exclusive and unique. By varying the concentration of the polymer different types of fruit gels were obtained with different characteristics. Research also showed that Gellan gum gels provided exceptional flavour release and had the ability to set rapidly (Saha & Bhattacharya, 2016). On the other hand, a contrasting study mentioned the importance of the polymer Gellan gum because of its significance in numerous food formulations. It has been used in jelly, jams, desserts, batters and even confectionary items due to its gel strength capability. It was also found that a higher concentration of Gellan resulted in very clear and transparent gels for example if it was 15% and above this was the case and below this concentration the transparency had reduced (Bell et al. 1993).

Gums have been used as ingredients to decrease calories and the fat content in a variety of food preparations since the 1980s. Moreover, gums in particular have good properties such as having the ability to control the viscosity of aqueous media and also aid in suspending

particles. Guar and xanthan gum have been commonly used as part of fat reducing food items. (Zambrano et al. 2004) carried out a study on these two polyelectrolytes in an attempt to produce low fat cakes.

Another study on Gellan gum in particular has been used for tissue engineering purposes with a bio-active glass particle. The results indicated that this polyelectrolyte helped in improving the overall structure and its physicochemical properties (Gantar et al. 2014).

Assessment of polyelectrolyte interactions

A number of methods have been applied to explore the interactions between polymers in particular ionically charged polymers. Factors such as turbidity, viscosity, NMR, thermal analysis, XRD, pH, weight ratio of polymers, UV spectroscopy are some methods that can be used in assessing the polyelectrolyte complexation process (Lankalapalli & Kolapalli, 2009).

This study will focus on the use of three factors turbidimetry, viscosity and zeta potential analysis to determine the ideal complex from a number of complexes at different concentrations.

- ### Zeta potential

The zeta potential is also known as the electrokinetic potential. This is the measurement of a particle moving under an electrical field. It can measure polycationic and polyanionic materials and gives results with a number value of how positive or negative it is (Bhattacharjee, 2016). A study reported the zeta potential results of alginate and tanfloc polyelectrolyte complexes. It was found that the ideal complexes had values of 12.7mV and 7.69mV. The results indicated cationic characteristics implying the tanfloc dominating the complexes (Facchi et al. 2017).

- ### Viscosity

The viscosity is a measure of how much resistance a sample can give when stress is applied (Oxford, 2016). (Zhang & Jia, 2006) stated that an ideal polyelectrolyte complex should have a low value for the viscosity to be able to process at a quicker rate.

- **Turbidity**

The turbidity of a solution is when a sample is not completely pure or clear and there is some amount of cloudiness (Oxford, 2017). A sample becomes turbid when an insoluble fragment or the complex itself reduces light (Aulton & Taylor, 2018). Turbidity was caused between the two polymers because a crosslinking reaction had taken place the positive and negative ions linked to form the complexes.

Polymers

Eudragit E100

Eudragit E100 is a colourless polymer and has cationic (positive) properties in the form of spherical granules and has a pungent amine like smell was introduced in the year of 1961 (Evonik, 2017) (Nikam et al. 2011). There are many different forms of Eudragit, and each of those have different uses. Studies specified that various types of Eudragit have been investigated extensively for controlled drug delivery systems. Eudragit is a polymer which is highly reliant on pH characteristics if it is being used as a polyelectrolyte in specific. For instance, it has the ability to swell yet remain insoluble in the gastrointestinal tract or intestines instead of being a normal soluble drug with a faster release rate than required. Eudragit types E, NE, RS and RL are all positively charged however, types S and L are anionic meaning that Eudragit has very useful properties (Moustafine, et. al. 2005).

Eudragit being a multipurpose polymer has the ability to be designed and adjusted according to the formulation requirements for example, a sustained release, delayed release or a rapid release can be prepared by knowing more about the functional groups found in the polymer structure and then tailor it according to the required type of formulation. Furthermore, Eudragit can also be used as a single coating layer or even as numerous coating layers. This is an advantage to pharmaceutical industries in particular because specific targeted locations for drug delivery require a precise drug just for that purpose. This specific polymer is of wide use in drug formulations because of its compatibility with other pharmaceutical excipients, which makes it possible to manufacture almost any type of drug (Evonik, 2015).

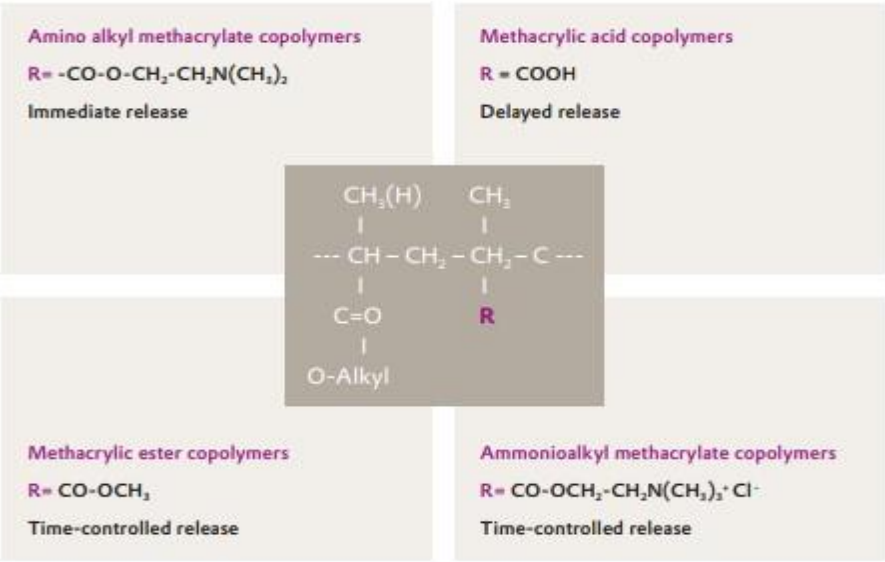

Figure 3- Structure of eudragit

Figure 3 shows the Eudragit polymer existing in different forms according to the forms of release it can conduct. Advantages such as versatility, compatibility with other excipients, high compressibility, stable thermally, capacity to produce large yields and it performs as a good adhesive are all beneficial to pharmaceutical industries (Evonik, 2015).

- **Chemical structure and properties**

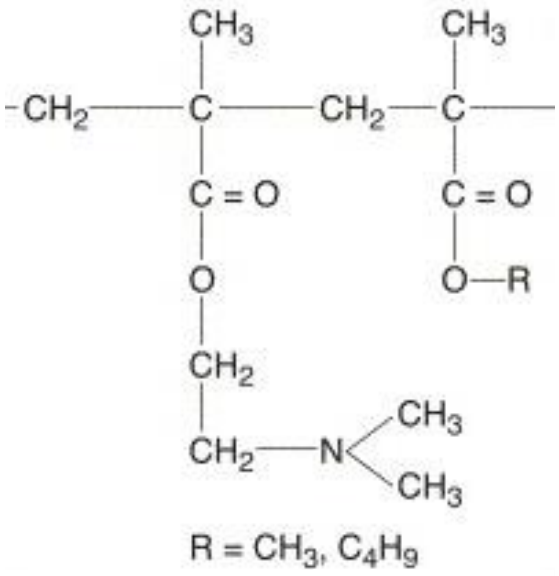

Figure 4- Structure of eudragit

(Moustafine, 2005) EE100

Eudragit E100 is a polymer made from a combination of methacrylic acid esters and dimethylaminoethyl methacrylate esters. It is only soluble in media such as gastric fluid or in solutions up to pH 5 and not more. Also, this polymer has found to be effective in taste masking drugs to bypass the bitterness. This added layer of coating also enhanced the dissolution rate in acidic media. Another beneficial property of Eudragit E100 is that the swellability of the formulation can also be altered because this polymer has the potential to modify the pH being soluble at a low pH. This factor would also affect the dissolution rate of the active entrapped inside and the solubility (Obeidat et al. 2015).

- **Pharmaceutical uses**

Polymers such as Eudragit have demonstrated to be very effective and useful in the pharmaceutical field. These polymers are largely used as part of tablet formulations and oral capsules in the form of a coating layer to provide the drug formulation a defensive layer to keep the contents within intact and deliver a sustained release profile. An example study was carried out between PEG 400 and Eudragit RS complexes to observe the thermal properties of it. The results indicated that the active drugs which were incorporated into the complex had become more permeable and increased in temperature above the glass transition resulting in a higher absorption of water due to the change caused by the inclusion of Eudragit RS. As the permeability had increased the rate of swellability also increased meaning that these polyelectrolyte complexes were very efficient biologically (Fujimori et al. 2005).

Another study illustrated the outcomes of a complexation reaction between Eudragit L100 and Eudragit E100 with the inclusion of Ibuprofen as the active drug. The results indicated a polyelectrolyte complexation being formed at pH 6, the reason being was because Eudragit E100 is only soluble at this pH. Nevertheless, Eudragit L100 is also soluble at pH 6 therefore, complexes were formed at this pH only. The results showed that the complexes swelled rapidly in an acidic environment but did not swell as much in a basic environment. This was due to the fact that Eudragit L100 is able to ionise its carboxylate groups upon hydration and Eudragit E100 did not ionise its dimethylamino groups. This study gave promising results to further explore controlled drug delivery formulations mainly because of the swelling data achieved (Moustafine et al. 2005).

Gellan gum

Gellan gum is a very significant polysaccharide, it is highly important in a wide rage of fields because of its versatility. It is anionic (negative) in nature and is derived from a bacterium identified as Sphingomonas elodea (Giavasis, et. al. 2000). (Smith et. al. 2007) specified that Gellan gum is a hugely effective and successful biopolymer because of its biodegradability and compatibility with many other polymers. It is also very well-known because of its tremendous use in the food industry and is now also being used in fields such as tissue engineering and various medicinal uses such as the treatment of cancer, HIV and fungal infections. In addition to this, Gellan gum is a popular polymer used as a thickener in food products such as jellies because of its gelling ability (Oxford, 2014). Applications within production businesses such as the pharmaceutical, cosmetics and food have taken used this polyanion to further explore and incorporate into medicinal formulations, food items and cosmetics. Gellan is a linearly structured polymer chain with tetrasaccharide repeating units. The repeating units comprise of D-glucose, D-glucuronate and L-rhamnose. Research specified that the largest amount of investigation and analysis had been carried out on Gellan in the food industry but over time it has developed to be a part of pharmaceutical formulations and has proved to be beneficial. It was found that Gellan behaved in various ways according to the type of media they were put in hence why, controlled release formulations were developed because they were able to be tailored to the required final product (Osmalek et. al. 2014). Over recent times extensive research has been carried out for chronic illnesses and obesity due to unhealthy eating and the consumption of sugary or high fat content foods. A number of products based on gellan have been developed for the replacement of such food items. Gellan is a hydrocolloid that has been part of the development of alternative and healthier food option. It was recently legalised by countries such as the USA and Japan to be used as an additive (Milan & Mileki, 2012). Furthermore, gellan is often used in food such as jams and jellies (Popa et al. 2004).

- **Chemical structure and properties**

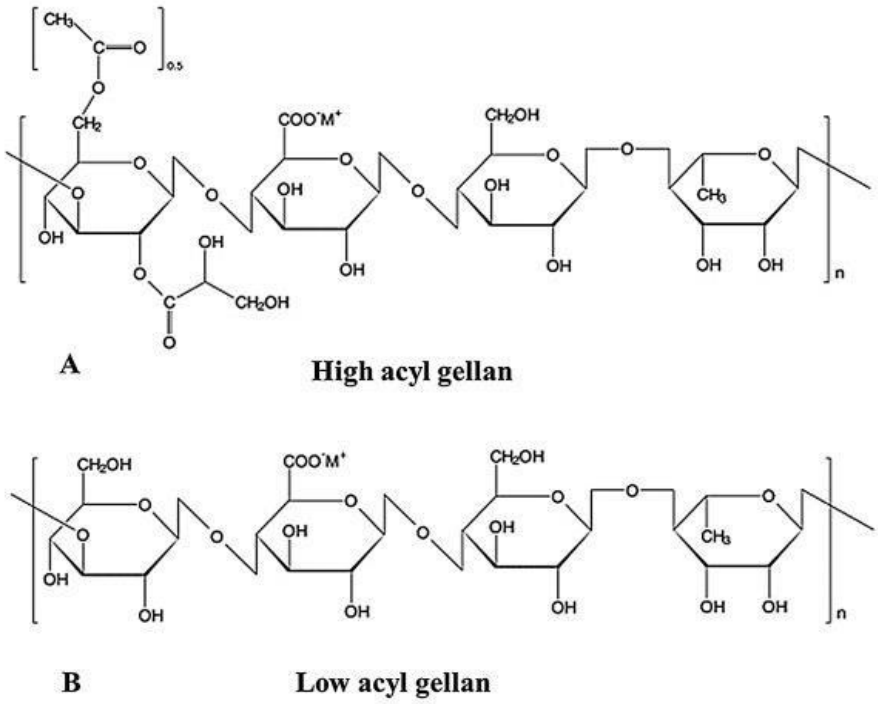

Figure 5- Structure of gellan gum (high and low acyl)

(Mahdi et al. 2015)

Table 2- Properties of gellan gum

Properties	Low Acyl Gellan Gum	High Acyl Gellan Gum
Hydration Temperature °C	45-90	65-90
Gel Set Temperature °C	35-55	>55
Viscosity (Hot) (1% 80°C)	<25	>100
Melting Range °C	80-140	71-75

(Pharmaceutical Excipients, 2017)

Table 2 displays the structure of high and low acyl Gellan gum chemical structures. The differences can be observed, the higher acyl gel having a larger composition in comparison to the low acyl gel. High acyl Gellan Gum comprises of 2 acyl groups (glycerate and acetate). Both the groups can be removed by a process including the use of hot alkali. This type of gel can form soft and viscous hydrogels at 65 degrees. On the other hand, low acyl Gellan Gum is a deacylated form of high acyl gel but has different physicochemical properties such as forming a hard and brittle hydrogel at 40 degrees whereas, the other forms soft gels (Zia et al. 2018)

- ## Pharmaceutical uses

Gellan has previously been used as part of oral drug delivery systems in the form of an excipient for sustained release formulations. Such types of preparations were tested and were found to have a higher quantity of Gellan Gum due to its swellability characteristics however, this is a critical and significant factor for the formulation to work efficiently and effectively (Morris et. al. 2012). Low acyl Gellan has the potential to go through a sol-gel transition phase hence, why oral drug delivery systems were studied broadly and turned out to be a better option for geriatric and paediatric patients because of the ease found in swallowability. Moreover, it was also seen that Gellan in the state of a liquid form presented a sustained release profile within the stomach (Miyazaki et. al. 1999). At a temperature of around 85 degrees Gellan is structured as random coils, these coils then form into helices when cooled because of a weak gel phase (Norton et. al. 1984).

It was also found that Sodium Alginate in the state of a liquid had a sustained release profile within the stomach, the reason for this was because sodium alginate behaves similarly to Gellan because of its ability to thicken and create a raft in the stomach which is a gel like layer and has the ability to release drug over time. An example of this would be Gaviscon for the treatment of heartburn (Miyazaki et. al. 1999). Gellan performs differently in different pH's, the results indicated that there was an increase in drug release at pH 2 in comparison to pH 6.8 (Alhaique et. Al. 1996).

Aim /objectives

Aim- To prepare and characterise PECs formed from EE and GG in order to achieve an ideal complex for further research.

- To determine the optimum weight ratio and explore factors effecting PECs
- To use a variety of analytical techniques to investigate and analyse differences between physical mixtures of polyelectrolytes and complexes
- To incorporate an active drug into the polyelectrolyte complex

Chapter 2: Materials and Methods

Materials

Low Acyl Gellan gum – purchased from Special Ingredients 500g, Chesterfield, UK, Eudragit E100- Evonik Industries AG, Germany 500g, Propranolol Hydrochloride ($C_{16}H_{21}NO_2$) MW= 295.81g/mol, Ibuprofen-(20(4-Isobutylphenyl) propionic acid ($C_{13}H_{18}O_2$) MW= 206.29g/mol, Acetic Acid 1M- Fisher Scientific, Hot plate stirrer, Mettler Toledo DSC STAR system, Mettler Toledo TGA 1 STAR system, FT-IR NICOLET 380, Jeol JSM-6060CV SEM, Bruker D2 Phaser XRD diffractometer, IKA RW 20 Overhead stirrer, Eppendorf Centrifuge 5702, Centrifugal Retsch Mill, Vacuum Oven, Malvern Zetasizer, Brookfield Viscometer, Jenway 7305

Spectrophotometer, Sieve shaker, VWR Symphony SB70P pH meter, sodium phosphate monobasic anhydrous form, sodium phosphate dibasic dihydrate (ACROS, ORGANICS) and sodium hydroxide pellets (Fisher Scientific, UK) for preparation of the buffer solution, Dissolution Apparatus, Dietmar Schulze Ring Shear Tester (RTS-XS), Turbula Mixer (Type T2 C), BVA Hydraulic Press

- **Calibration Curves**

Pure forms of active drugs Propranolol and Ibuprofen were weighed to a constant value of 100mg using an analytical grade balance. Ibuprofen having poor solubility properties had to be dissolved with 2mL of methanol. Concentrations of 1mg/mL were made for both of the drugs with 0.1M pH 6.8 phosphate buffer or 0.1M HCl. This was used as the main stock solution from which several different concentrations of 1, 2, 5, 10, 15, 20 and 25mL were taken and prepared according to the correct volumes. These were then measured in a quartz cuvette in a UV spectrophotometer thrice, the absorbances were recorded an average was taken. Propranolol was measured at a wavelength of 289nm and Ibuprofen was measured at a wavelength of 222nm.

After the measurements were done, calibration curves were plotted on Excel, the absorbance against concentration charts were made and then the linearity was determined from this.

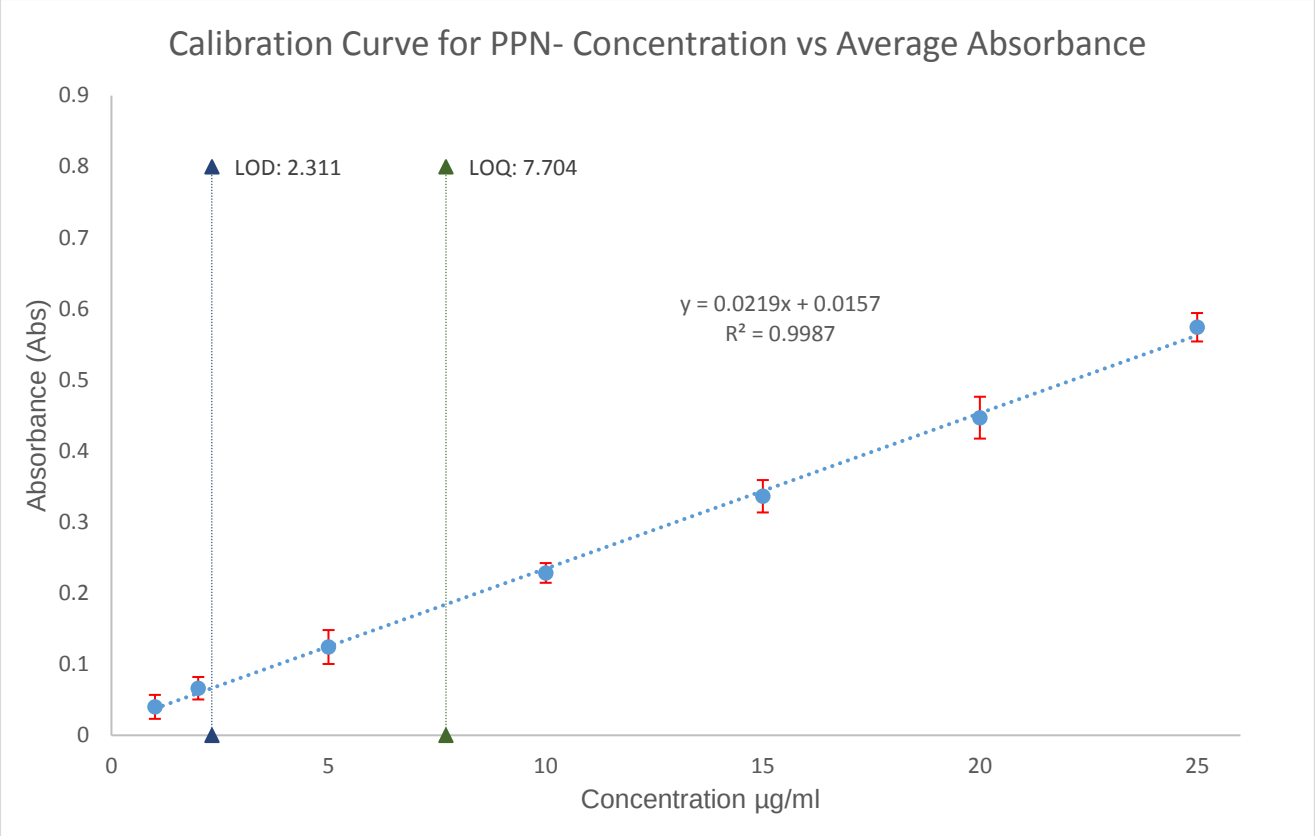

Figure 6- Calibration curve of PPN in buffer

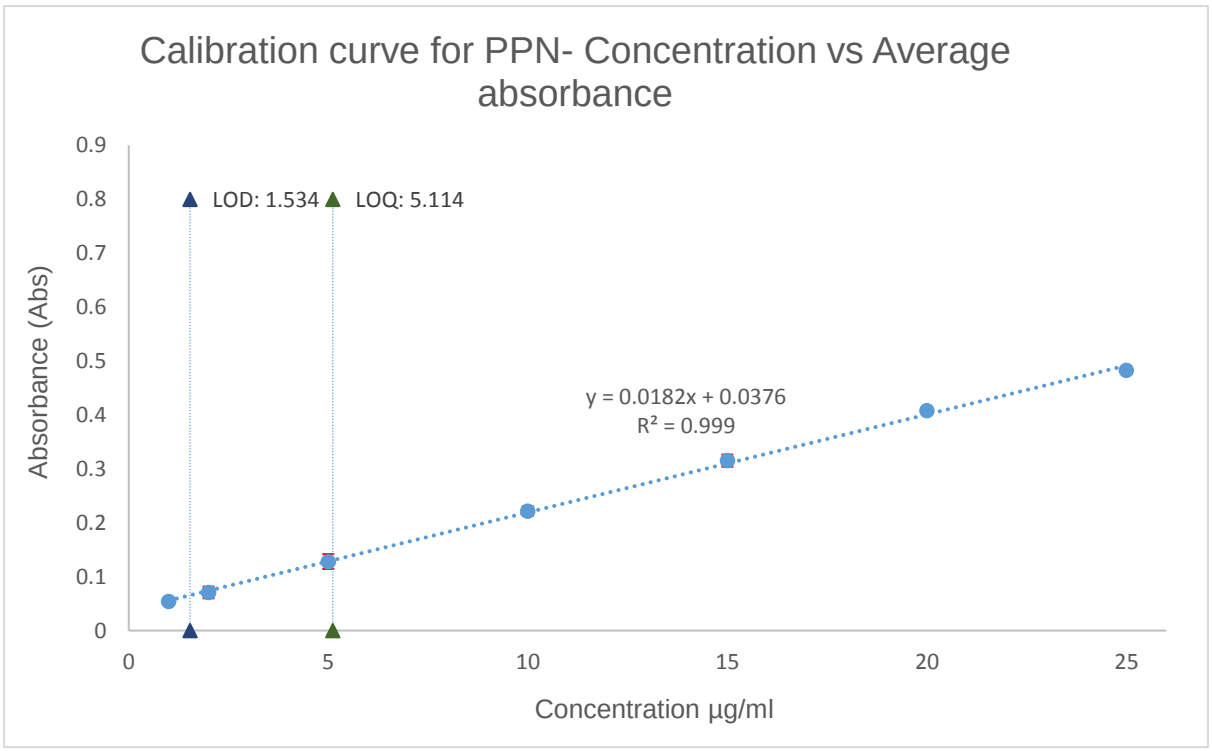

Figure 7- Calibration curve of PPN in HCL

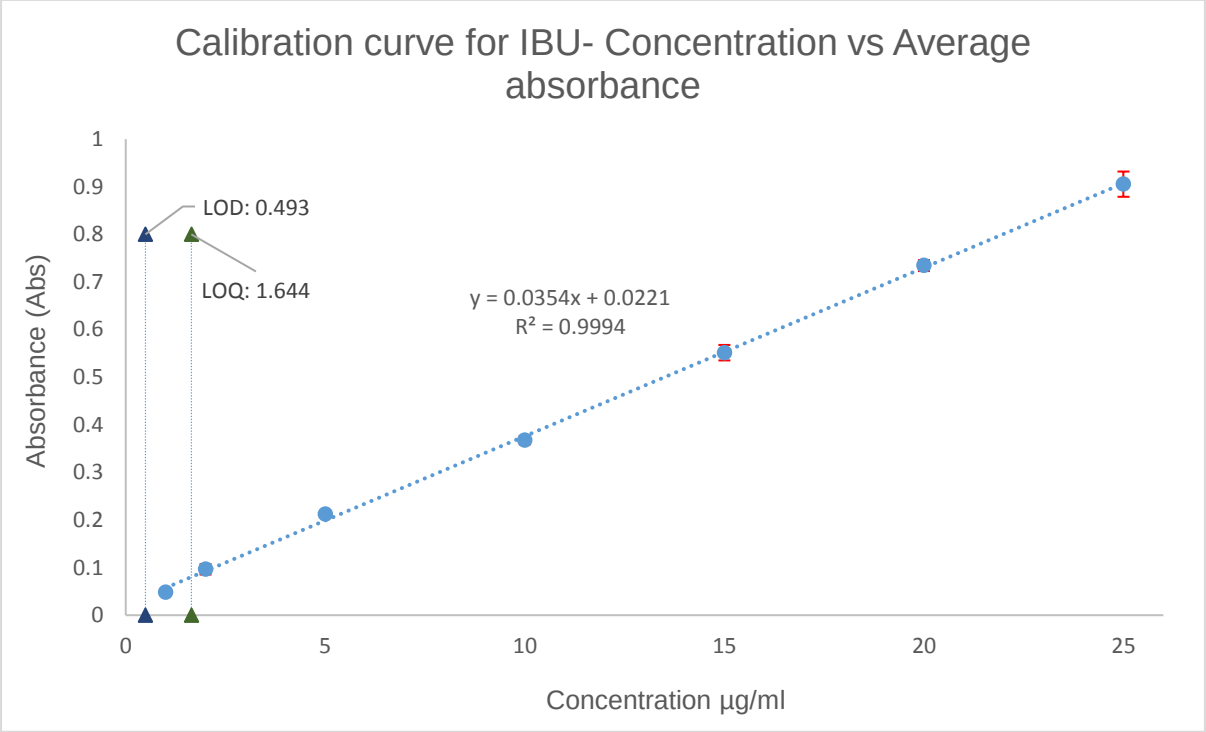

Figure 8- Calibration curve IBU in buffer

- **Polymer solutions preparation**

Optimisation Stage: 0.5g of Gellan gum (low acyl) was dissolved into 100ml of deionised water at 85 degrees to produce a polymer solution of 0.5% concentration and was then cooled at room temperature. Similarly, 0.5g of Eudragit E100 granules were dissolved in acetic acid (1M) without heat to again produce a 0.5% polymer solution.

Scale-up Stage: Larger volumes of the polymer solutions were prepared by dissolving 25g of Gellan gum into 5 litres of deionised water at 85 degrees. In addition, 25g of Eudragit E100 granules were dissolved into acetic acid and deionised water. 287.5 ml of acetic acid was used and then water was added to make the volume up to 5 litres. Both polymers were left on a hot plate stirrer for uniform mixing until completely dissolved on 500rpm.

Table 3- Concentrations of GG:EE polymers

100mL samples		
Weight Ratio	GG (mL)	EE (mL)
0.20	16	84
0.50	33	67
1.00	50	50
1.25	55	45
1.50	60	40
1.75	64	36
2.00	67	33
5.00	84	16

- **Complexation Reaction (Optimisation Stage)**

The complexation reaction was done by accurately measuring the required amounts of polymer solutions separately into beakers. For example, 50ml of Gellan gum and 50ml of Eudragit E100. For this complexation Gellan was added to the Eudragit solution while it was mixing at 1000rpm with an overhead stirrer dropwise for 60 minutes. This method of complexation is known as a titration method. After an hour of agitation, the stirrer was stopped and the polymer complexes were covered with parafilm and left for 24 hours in a fridge for the complete crosslinking.

The complexes were divided into Eppendorf tubes for centrifugation for 40 minutes at 4400 rpm. Two layers were present in the tubes one being the supernatant which was collected for further tests and the sediment was collected and washed with deionised water and centrifuged

again for a further 10 minutes to obtain a purer sample. The supernatant was used to test the viscosity and the zeta potential.

- **Drying and Sizing of the complexes**

The wet sediments were dried using the oven at 40°C for 48 hours until completely dry. The complexes dried in the form of brittle and thin films. To make sure the weight was constant the samples were weighed at regular intervals to maintain a constant weight.

The dried samples were milled down to 125µM using the centrifugal Retsch mill at 10,000 rpm for 5 seconds and stored separately at room temperature for further analysis.

- **Drug entrapped complexation**

Three different drug concentrations were chosen to perform the drug entrapped complexes. This was done to determine the properties of the complex as a matrix and the drug release profile in order to be able to know how much drug is releasing over a certain time. The same method as mentioned above was repeated but with the addition of drugs. For example, 1g of propranolol was added to 33ml of 0.5% Eudragit solution, this was then placed in an ultrasonic bath for 15 minutes to ensure the removal of contaminants. 67ml of 0.5% Gellan gum solution (room temperature) was added dropwise to the propranolol + Eudragit solution and was stirred for 20 minutes at 1000rpm. The final concentration of the solution was 1% (1g propranolol in 100ml). The polymer-drug-polymer complex was left for 30 minutes for the crosslinking to happen. The same method was applied for all the different drug concentrations and polymer concentrations. In this study propranolol (cationic drug) was added to Eudragit (cationic polymer) and Ibuprofen (anionic drug) was added to Gellan gum (anionic polymer). In contrast to propranolol, 1g of Ibuprofen was added to 33ml of Gellan gum solution and was added dropwise to 67ml of Eudragit.

- **Zeta potential**

The supernatant of the polyelectrolyte complexes was placed into a cuvette and a dip cell probe was inserted. The sample was placed into the Zetasizer and measured the Zeta potential for the same sample three times and gave an average measurement.

- **Viscosity**

The Brookfield Viscometer was used to measure the viscosity of the individual supernatants of the polyelectrolyte complexes with the use of the correct spindle attachments. The spindle was immersed into the liquid pre-measurement to observe the viscosity (cP) at 100rpm until the value was stable.

- **Turbidimetry**

The turbidity was measured using a Jenway 7305 Spectrophotometer at various wavelengths depending on what was being measured. For example, pure polyelectrolyte complexes were measured at 560nm but drug entrapped polyelectrolyte complexes were measured at a wavelength of 289nm for Propranolol and 222nm for Ibuprofen. Each sample was placed into a quartz cuvette and measured after calibration.

- **FTIR**

The powder samples were placed on to the FT-IR Attenuated Reflection plate to distinguish the different functional groups present within the polymers, polyelectrolyte complexes and drug entrapped complexes. Around 10mg of powder samples were analysed at a wavelength from 400-4000cm^{-1}. Infrared correlation tables were used to determine functional groups, bends and stretches present to confirm any changes observed.

- **Thermal analysis**

DSC

DSC analysis was performed using the Mettler Toledo DSC 1 apparatus. 40 microlitre Aluminium crucibles were used to perform the test. Powders between the ranges of 5-10mg were prepared and weighed with a hole at the top of each pan before being placed for analysis. A Nitrogen purge of 50mL^{-1} over a heat range of 25-300°C at 10°C min^{-1} was used as the method. The same method was applied for all of the samples.

TGA

TGA analysis was performed using the Mettler Toledo TGA STAR 1 system. 40 microlitre Aluminium crucibles were used to perform the test. Powders weighing between the ranges of 5-10mg were prepared with a hole at the top of each crucible before being inserted for analysis. A Nitrogen purge of 50mL^{-1} over a heat range of 25-450°C at 10°C min^{-1} was carried out. The same method was carried out to test the other samples.

XRD

The XRD data patterns of the polymers and complexes were tested to determine whether they crystalline or of amorphous nature using the Bruker D2 Phaser XRD diffractometer apparatus. The powder samples were placed onto a plastic disc carefully before being inserted into the machine. Each sample was analysed over a 4-minute scan from 5°-100° 2θ at a rate of 11° min^{-1}.

SEM

The SEM was used to observe the polyelectrolyte complexes, pure polymers and beads in more detail and at different magnifications. The Jeol JSM-6060CV SEM was used to observe particle characteristics such as shape, size and texture under various magnifications. The samples were prepared individually by scattering a minute quantity of powder/bead onto a sputter with a gold: palladium coating for at least 60 seconds with the use of the Quorum SC7620 sputter coater.

Shulze Shear Cell

The Shulze shear cell was used to test the flowability of the dried powders. This was done by using a software called RST-Control, the order of the program was set to SS and the shear cell setting was put onto number 2. Dried forms of the powders were analysed for powder flowability and the bulk density. Firstly, the powder was placed onto the ring without using any compression, this was weighed before and after every reading without the top. The loading rod and tie rods to help keep the ring in place were attached to the ring then the procedure had started the analysis.

Tabletting

The BVA Hydraulic press was used to compress the powders into tablets. The appropriate punches and dyes were chosen (5mm) and used to prepare the compacts. Each powder was weighed individually according to the amount required and poured into the dye. A pressure of 1000 psi was used for every formulation and a dwell time of 30 seconds was carried out for each tablet consistently before the tablet was ejected. This process was repeated for all of the formulations in the same way.

Drug release

The drug release of the active drugs from the matrices were calculated by adding 5mg of milled polymer drug complexes into either 50 ml of 0.1M HCl or PBS pH 6.8. The different concentrations were weighed out and placed individually for stirring at 600rpm for 24 hours. Measurements were taken 24 hours later at their respective UV wavelengths in a spectrophotometer and noted. Furthermore, these values were used to calculate the drug release using the initial calibration curves.

Dissolution

The dissolution testing was carried out of the tablets containing the active drugs and without the drugs using the manual dissolution apparatus. Both of the drugs Ibuprofen and Propranolol polymer complexes and the physical blend forms of tablets were tested in 0.1M HCl for up to 2 hours and in PBS pH 6.8 for 8 hours. Readings were taken at set intervals and read using a spectrophotometer at their individual wavelengths. All of the experiments were carried out at 37 degrees Celsius at 50rpm.

Ionotropic gelation method

The beads were dropped into the calcium chloride solution dropwise through a syringe while on a hot plate stirrer. They were kept on stirring for 30 minutes to ensure they crosslink properly and form hard beads.

Chapter 3: Polyelectrolyte complexation optimisation and characterisation

Introduction

This chapter consists of how the polyelectrolyte complexation was optimised and narrowed down the final concentrations that will be examined further with numerous laboratory-based techniques and methods. Furthermore, the final concentrations will be characterised and used as tablet matrices with and without active drugs.

Determination of optimum complexation ratio

The optimum complex was determined via three techniques (turbidimetry, viscosity and zeta potential) that are used to establish polyelectrolyte complexation reactions including the ideal complex.

As the reaction was carried out between the two polymers a cream/white solid like precipitate had formed while it underwent a spontaneous reaction. This reaction was carried out under constant agitation at a speed of 1000rpm for one hour before isolating them for 24 hours for full complexation.

- **Turbidity**

The turbidity of a solution is when it is not entirely clear or a 100 percent pure and a quantity of cloudiness is present (Oxford, 2017). A sample is found to be called turbid when fragments of insoluble nature or the complex itself can reduce light transmission (Aulton & Taylor, 2018). Cloudiness is caused when two polymers react together because of their positive and negative ionic charges causing a crosslinking reaction. The more turbid a sample, the higher chance of it entailing a higher amount of polyelectrolyte complexes.

Figure 9 shows the average turbidity of different weight ratio complexes. It was found that weight ratio 1.75 was the most turbid in comparison to the others. This was the concentration with 2 parts of Gellan gum and 1 part of Eudragit exactly. In addition, the results indicated that as the concentration of Gellan gum was increased the sample also became more and more turbid which specified and confirmed complexation was taking place more strongly.

Table 4 (refer to appendix) displayed the 1.75 weight ratio to have an average absorbance of 1.696, this was the highest amongst the rest indicating maximum turbidity and also meant that it was the most stable polyelectrolyte complex and could be concluded as the ideal complex. The graph presents a sharp peak at 1.75 to show this reasoning. Similarly, (Moustafine et. al. 2005) carried out an experiment between two polyelectrolytes Eudragit E100 and Eudragit L100 to determine the ideal complex. The results from the study showed that the 1:1 ratio exhibited maximum turbidity. Further to this, the results showed that the addition of more Eudragit E100 caused the complexes to segregate and lower the turbidity. This finding was similar to the study being carried out because higher amounts of Eudragit E100 had low turbidity levels. (Doi & Kokufuta, 2010) also found similar results from a study carried out between potassium polyvinyl alcohol sulfate and cationic nanogel. The highest turbidity was found to be for the 1:1 complex.

Table 4 also presents the absorbance values for all of the different concentrations being investigated in this study alongside values for the pure polymers.

Table 4- ABS of weight ratios GG:EE

Weight Ratio GG:EE	Absorbance 1	Absorbance 2	Absorbance 3	Average	SD
GG	0.015	0.012	0.015	0.014	0.002
0.20	0.209	0.215	0.221	0.215	0.006
0.50	0.863	0.861	0.870	0.865	0.005
1.00	1.432	1.438	1.435	1.435	0.003
1.25	1.440	1.444	1.449	1.444	0.005
1.50	1.495	1.503	1.500	1.499	0.004
1.75	1.695	1.703	1.690	1.696	0.007
2.00	1.424	1.420	1.419	1.421	0.003
5.00	0.997	0.980	0.987	0.988	0.009
EE	0.102	0.105	0.110	0.106	0.004

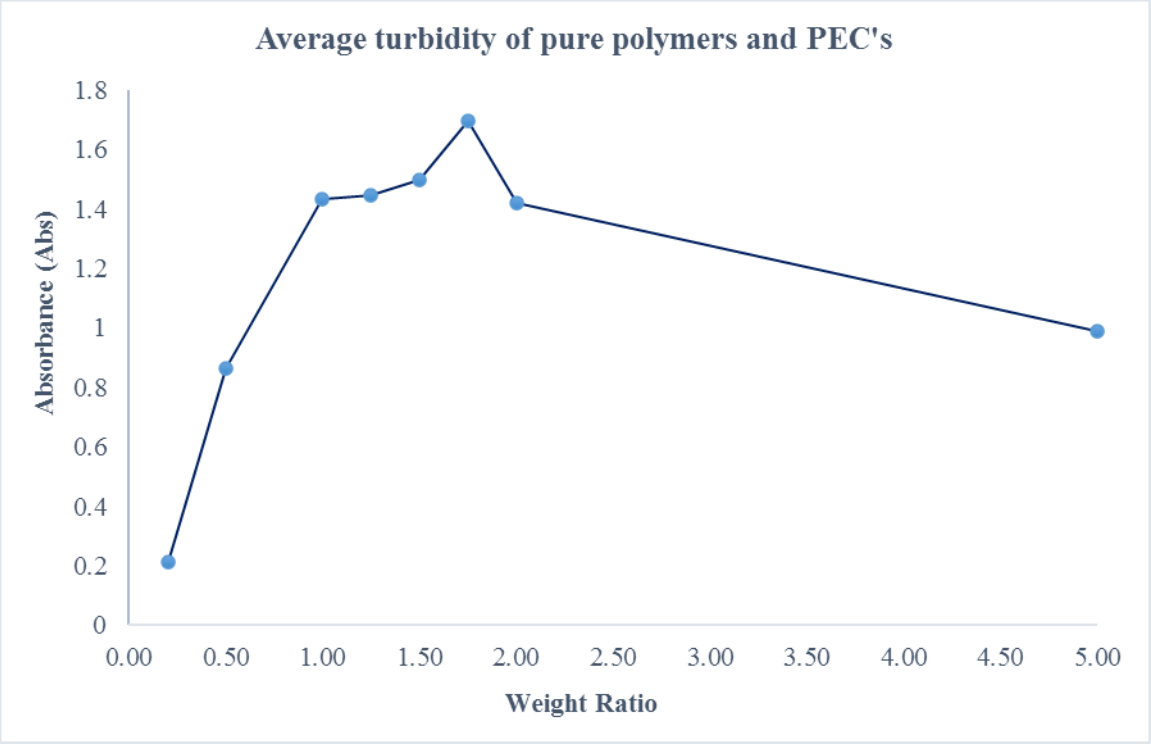

Figure 9- Turbidity of pure polymers and PECs

- **Zeta Potential**

The zeta potential is a measurement of the force of repulsion between ionically charged particles (negative and positive). Furthermore, it helps in determining the magnitude of how exactly how much repulsion is existent of the electrical double layer (Oxford, 2012). For an ideal polyelectrolyte complex the zeta potential should be balanced for example the ratio from cation to anion should be equal and the zeta value should be close or equal to zero. Figure 10 shows the average zeta potential of the different weight ratios being analysed and the pure polymers. The results indicated that weight ratio 1.75 which previously exhibited the highest turbidity had an average Z value of 10.5mV, which was the value closest to zero. The other concentrations had various fluctuating values in correspondence to how much of the polymers were present.

Reasons for the 1.75 concentration being closest to zero was because this was the weight ratio where it was nearly fully neutral ionically. Henceforth, it could be concluded that there was practically enough Eudragit to neutralise the Gellan gum. In contrast, this can be related to the turbidity as the 1.75 concentration was the most turbid. A model study conducted by (Kaur & Kaur, 2018) presented data achieved from zeta analysis from the reaction of

polyelectrolytes Gellan gum and cationic guar gum. The results showed that the ideal complex was found to be ratio 70:30 again being very similar to the experiment being carried out above. The zeta potential for this concentration was almost zero signifying optimum polyelectrolyte complexation reaction. Moreover, (Zhang et al. 2017) conducted a comparable experiment between polymers carboxy-methyl starch and chitosan. The results from this study portrayed similar zeta potential findings to Figure 10. It was established that larger concentrations of chitosan had positive Z values but the addition of carboxy-methyl starch shifted the positive Z value to negative.

Table 5 (refer to appendix) showed the average values obtained from the zetasizer apparatus alongside the pure polymers. Positive and negative values were found for different concentrations and the pure polyelectrolytes depending on their ionic charge.

Table 5- Zeta potential of pure polymers and PECs

Weight Ratio	Zeta Potential 1	Zeta Potential 2	Zeta Potential 3	Average	SD
GG	**-15.00**	**-16.30**	**-16.42**	**-15.91**	**0.79**
0.20	40.4	38.1	42.0	40.2	2.0
0.50	47.7	48.2	48.3	48.1	0.3
1.00	39.1	38.8	38.6	38.8	0.3
1.25	30.7	32.1	32.2	31.7	0.8
1.50	33.0	32.0	27.8	30.9	2.8
1.75	10.8	10.1	10.5	10.5	0.4
2.00	29.9	28.8	30.3	29.7	0.8
5.00	-19.2	-17.7	-15.7	-17.5	1.8
EE	**19.00**	**19.19**	**20.20**	**19.46**	**0.65**

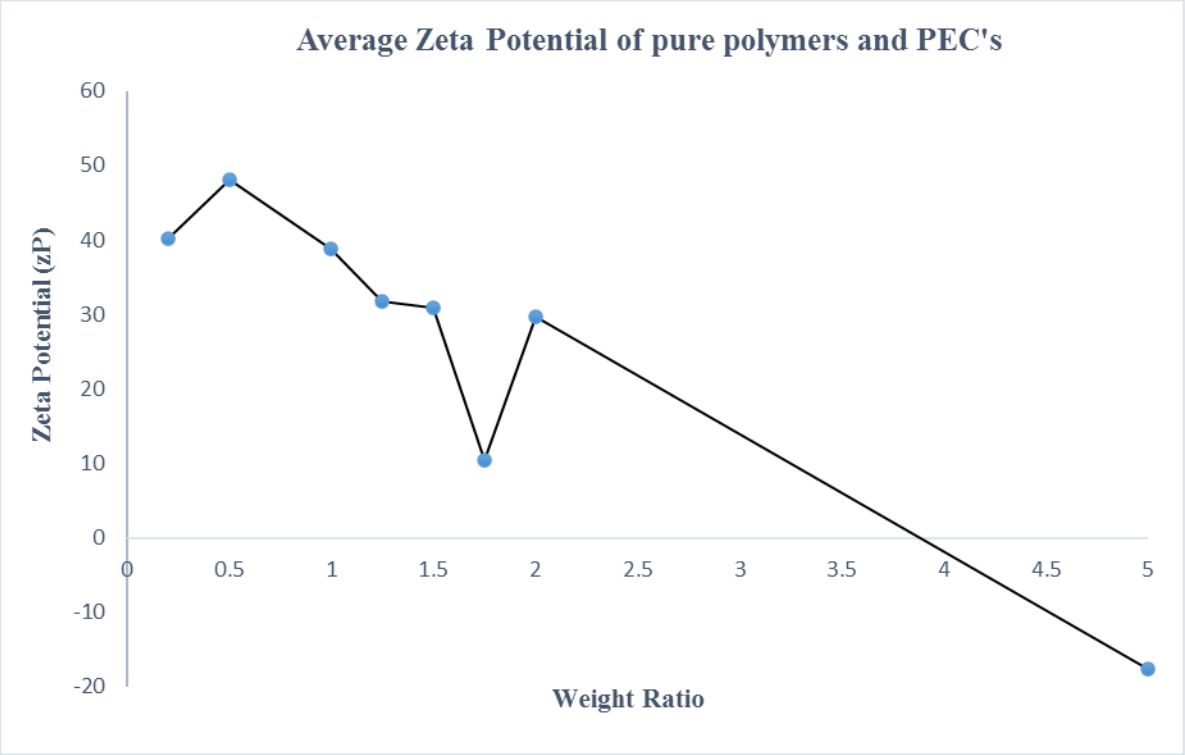

Figure 10- Zeta potential of pure polymers and PECs

- **Viscosity**

The viscosity is a numerical measurement of the total resistance that could be proposed when an amount of stress is applied to the medium being analysed (Oxford, 2016). Figure 11 displayed the average viscosity results achieved from the study and also the pure polymers at a constant speed and temperature. The results indicated that Gellan was very viscous in comparison to the complementary polyelectrolyte, Eudragit. In addition to this, the different polyelectrolyte complexation reaction supernatants were found to have much lower viscosities. The reason being was because these were supernatants only and not pure polymers but also because Eudragit had a very low viscosity itself. Figure 11 showed the trend of the different viscosities measured and also of the polymers being tested. The weight ratio 1.75 had an average viscosity value of 12.1cP which was also the lowest viscosity recorded from all of the others. The other parameters tested to determine the ideal complex (zeta potential and turbidity) fit into this trend and further confirmed 1.75 was the ideal concentration. A low viscosity for this concentration meant that the turbidity would have been the opposite result as in the highest, suggesting that the best polyelectrolyte complexation reaction had taken place for 1.75 and the supernatant for the viscosity was low because most of the polymers had reacted and were collected leaving a less viscous supernatant.

Gellan gum in the form of a solution is recognised to be highly viscous with a cP rate of approximately 272 (Chen et al. 2006). The viscosity is explained for Gellan as it undergoes a sol-gel transition stage upon hydration and Eudragit easily dissolves at pH 5 and below (Gao et. al. 2015). (Evonik, 2015) described how Eudragit had a viscosity value of 17.3cP, this finding reflects similar data obtained from Table 6. Moreover, research quantified that an ideal polyelectrolyte complex must have a low cP value so the it can progress at a more rapid rate in comparison to the other values obtained (Zhang & Jia, 2006). This further echoes the findings from Figure 11 and Table 6 because the viscosity was the lowest for weight ratio 1.75 in comparison with the others.

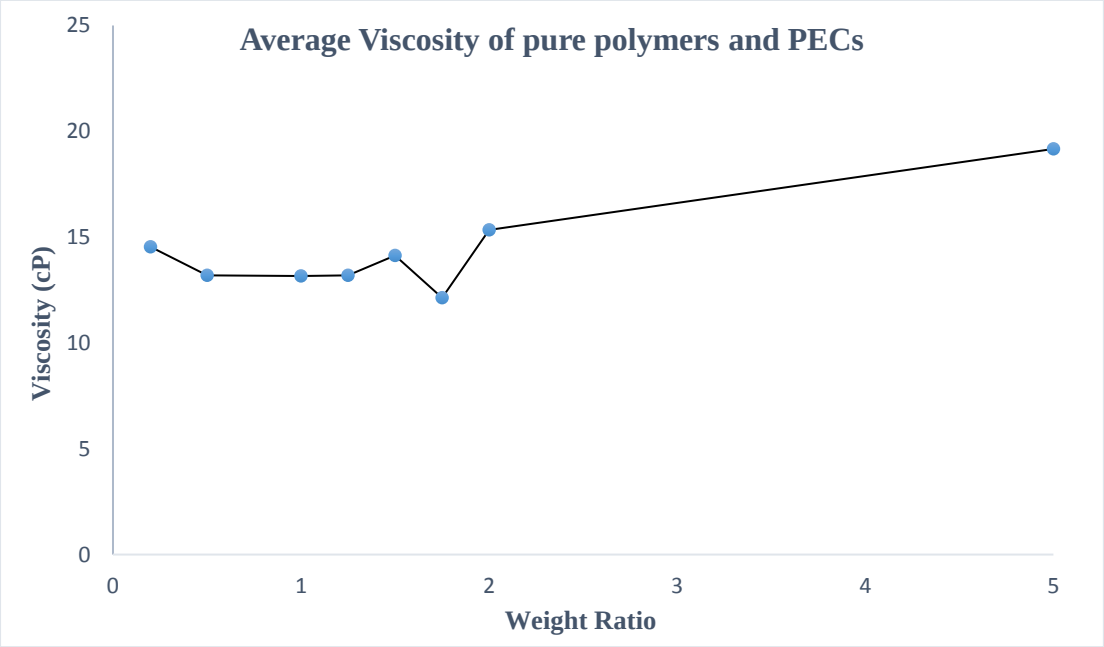

Figure 11- Viscosity of pure polymers and PECs

Table 6 Viscosity of pure polymers and PECs

Weight Ratio	Viscosity 1	Viscosity 2	Viscosity 3	Average	SD
GG	273	272	272.4	272.467	0.503
0.20	14.4	14.6	14.6	14.5	0.12
0.50	13.2	13.0	13.4	13.2	0.20
1.00	13.0	13.2	13.3	13.2	0.15
1.25	12.9	13.3	13.4	13.2	0.26
1.50	14.2	14.0	14.2	14.1	0.12
1.75	12.1	12.0	12.3	12.1	0.15
2.00	15.4	15.4	15.2	15.3	0.12
5.00	19.2	18.9	19.4	19.2	0.25
EE	18	17	17.6	17.533	0.503

Complexation characterisation

- **FTIR**

FTIR spectra displayed in Figure 12 showed the physical blends of the different weight ratios alongside the pure polymers. These blends illustrated minimal differences in their spectra, the overlapped lines indicated no reaction amongst the polyelectrolytes in the dry form powders only.

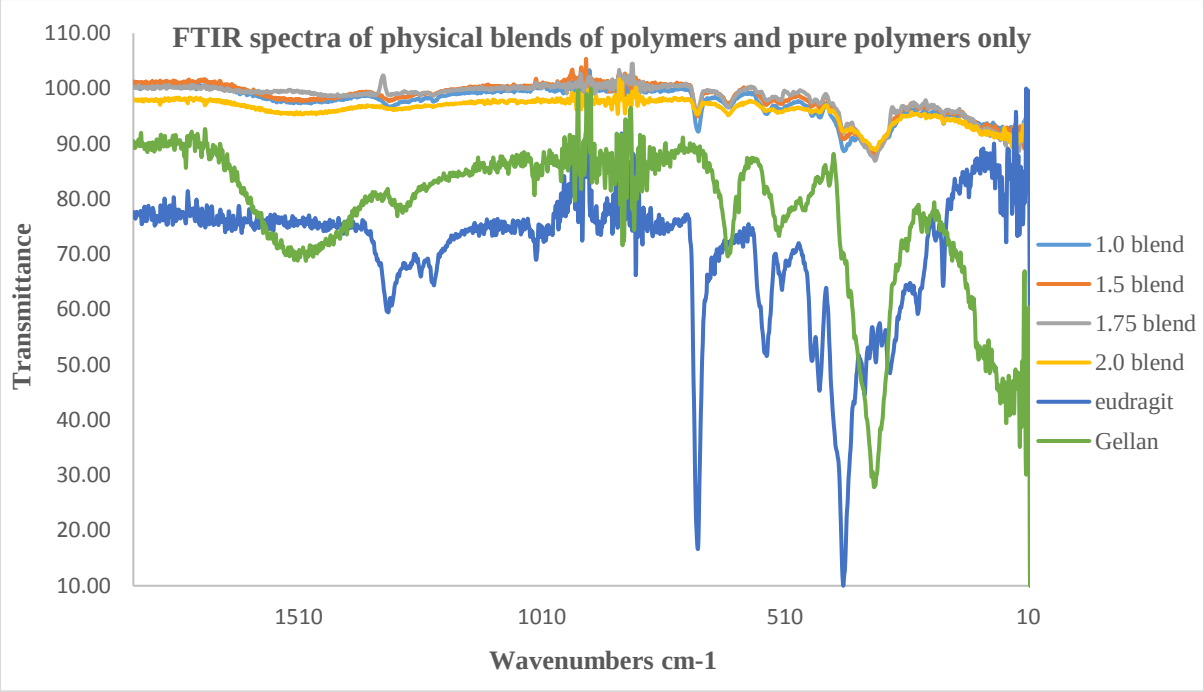

Figure 12- FTIR of blends of polymers and pure polymers

Figure 12 displayed the polyelectrolyte complexed samples and also just the polymers on their own. The data suggested that the newly formed complexes did not have major differences between them but the peaks either became broader and deeper or smaller and shallow. For example, 1.0 PEC the peak at approximately 1000 cm^{-1} is more distinct than the other concentrations because it has an equal amount of Gellan to Eudragit but this is not the case for 2.0 PEC where the peak is much flatter. This broad peak is visible in all of the spectra indicating C-O stretches. However, the most significant peak for the ideal complex weight ratio (1.75) was at 1721.0 cm^{-1} (possible amide). In addition to this, a peak at 1059 cm^{-1} (C-O stretches) was also existent in the physical blends but not present in the complexed spectra. A reason for this would be a strong indication of exactly where the complexation reaction had taken place between the polyelectrolytes. The identification of the functional group present was a carboxylic acid group which was the outcome of a di-methylamino group from Eudragit undergoing protonation (Moustafine et al. 2005). The polyelectrolyte complexes spectra illustrated various functional groups both belonging to Eudragit and Gellan polymers confirming the polyelectrolyte complexation reaction happening (Chakraborty et al. 2014). Another peak at 2946.9 cm^{-1} was present and was found to be a characteristic peak of Eudragit (Moustafine et al. 2006). In Gellan a broad peak was visible at 3410 cm^{-1}, this was identified as an O-H stretch which also reflects the spectra from the chart. Furthermore, Gellan also had

a stretch at 1601 cm^{-1} this being a COO group. Eudragit showed characteristic peaks at 1600 cm^{-1} and 1450 cm^{-1} these being the amides. It was found that both of these amide groups in the Eudragit polymer were not present in the complexed versions and a set of novel peaks appeared at 1400 cm^{-1} this is the area where the polyelectrolyte complexation reaction happened (Kaur & Kaur, 2018).

(Moustafine et. al. 2005) stated that there were two specific peaks allotted for alkane bending these were 1412 cm^{-1} and 1552.7 cm^{-1}. A study carried out by (Agarwal et. al. 2013) found that the infrared spectra for Gellan gum consisted of an O-H stretch at 3415 cm^{-1}.

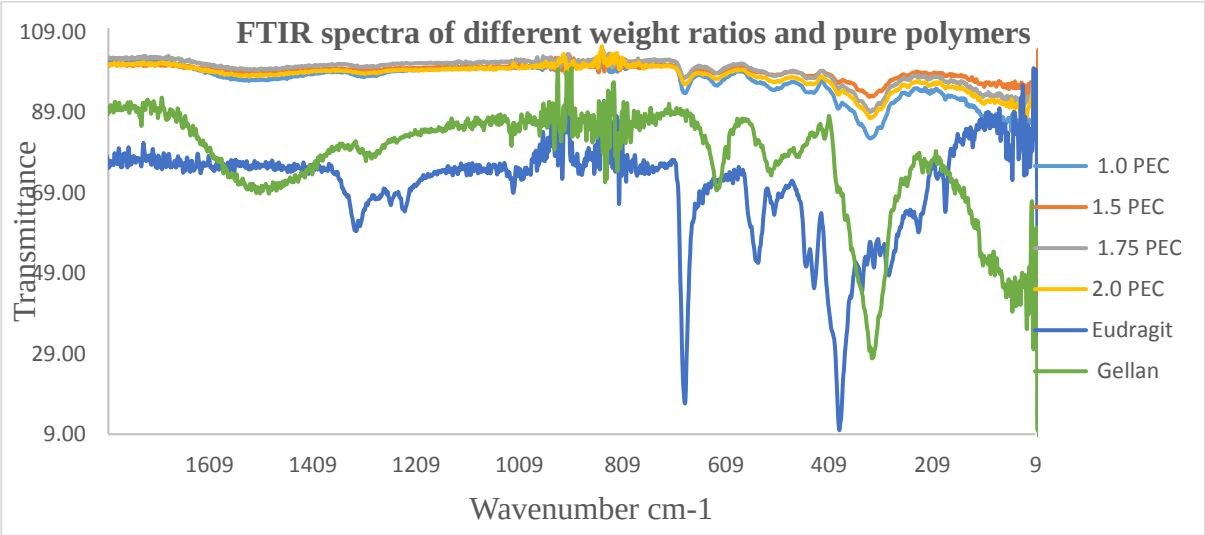

Figure 13- FTIR of complexes and pure polymers

Figure 14 displayed the spectra for propranolol and propranolol complexes 1% at different weight ratios. Firstly, the spectra showed the characteristic peaks of propranolol alongside peaks belonging to the polyelectrolytes Gellan and Eudragit. The drug complexes indicated different spectra because of the inclusion of the polymers. For example, the peaks at 1266.4 cm^{-1} proposed to be in all of the spectra including the pure drug showing to be an Aryl group (O-CH_2). In addition to this, (Chaturvedi et. al. 2010) stated that a Naphthalene group was present in the pure propranolol therefore also visible in the complexed forms at 796.9 cm^{-1}. Moreover, the intensity of the peaks varied amongst all of the spectra in Figure 14 which could be due its quantitative aspects.

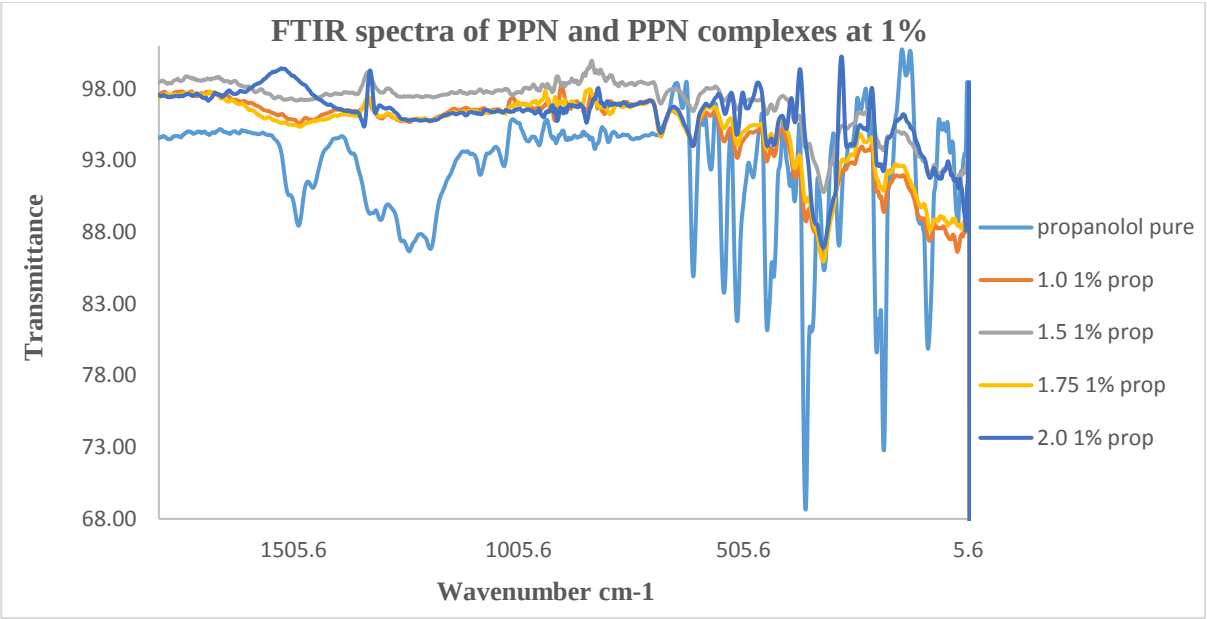

Figure 14- FTIR of pure polymers and complexes

Figure 15 displayed the spectra for Ibuprofen and Ibuprofen complexes 1% at different weight ratios. The presence of Ibuprofen was detected because of the broad peaks ranging from 2866.9 cm^{-1} to 2949.3 cm^{-1} (sp3 C-H stretches) represented the active drug. Peaks in this range were noticed in all of the spectra confirming drug entrapment within the complexes. Furthermore, a distinct peak at 2358.3 cm^{-1} was observed in the pure drug spectra but had completely disappeared in the drug complexes. Another prominent peak at approximately 1700 cm^{-1} (carbonyl group) was noticed in all of the spectra for this drug and drug complexes but the intensity of the peak had reduced in the complexed versions in comparison to the pure drug, this peak indicated a carbonyl group (Mallick et. al. 2011).

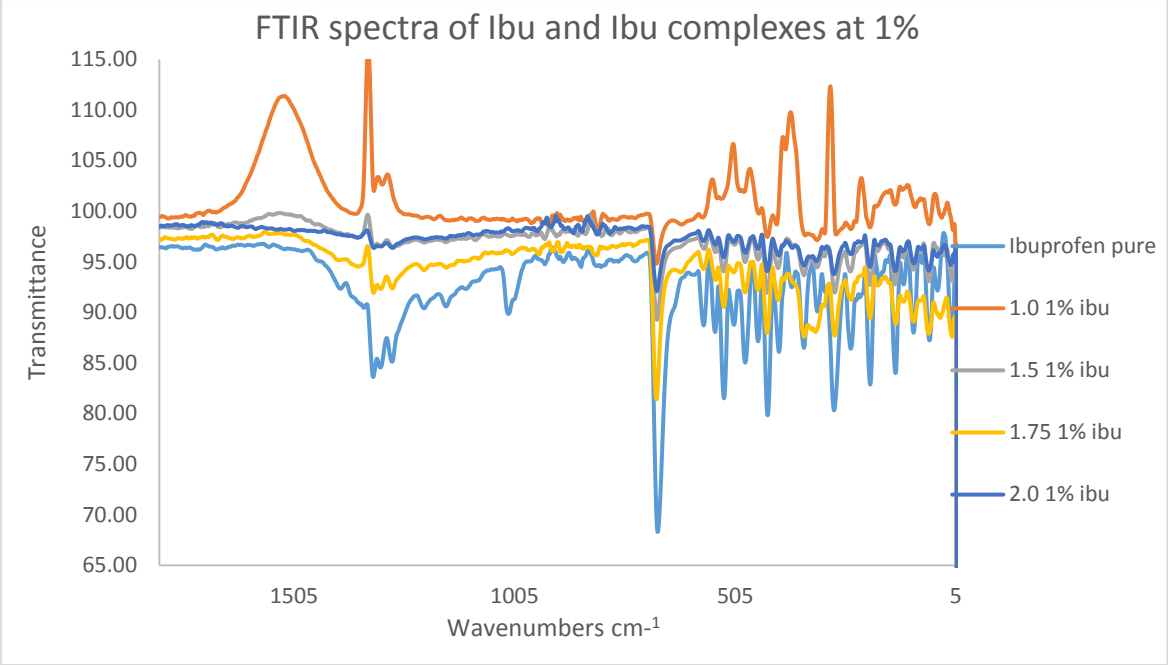

Figure 15- FTIR of complexes and pure IBU

Thermal analysis

- **DSC**

Figure 16 displayed the DSC data obtained from the physical blends at different weight ratios and also the pure polymers Gellan and Eudragit (y and z). At 255°C an exothermic peak was noticed for all of the blends including Gellan. However, this was not the case for Eudragit as no exothermic was seen.

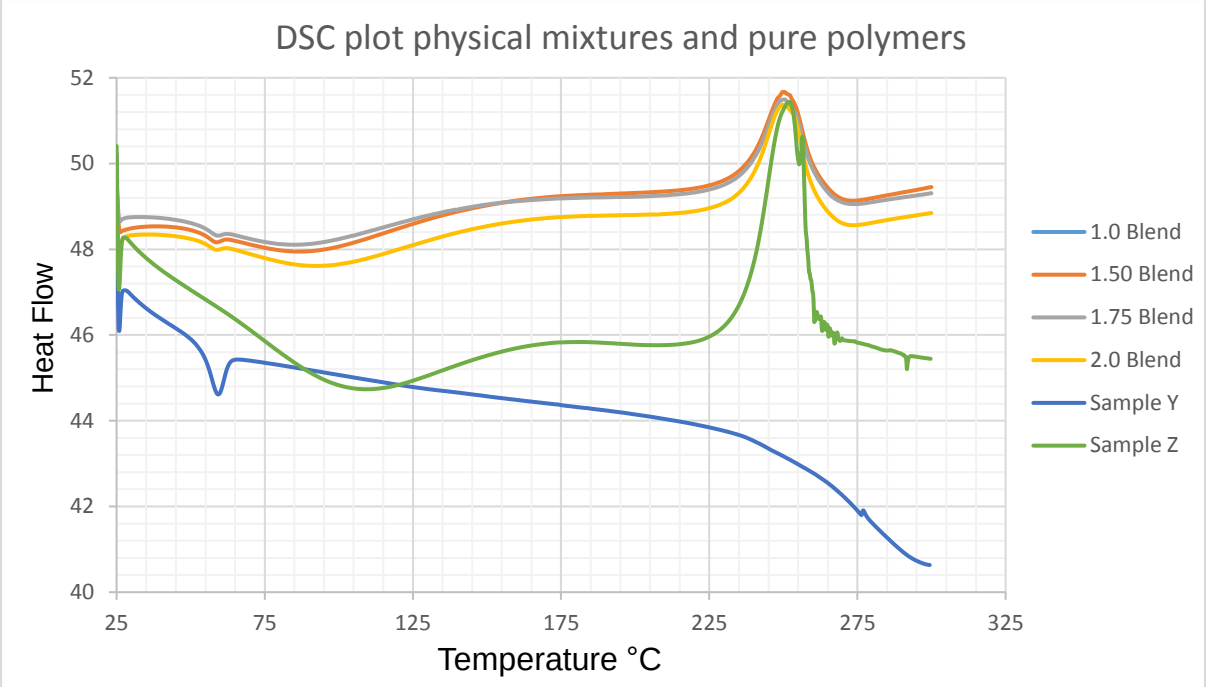

Figure 16- DSC of blends and pure polymers

Figure 16 showed the data obtained from the DSC analysis of the blank complexes and pure polymers. The results obtained from this showed a broad gellan peak at 85°C and carried on in this particular fashion until it reached 145°C, this is most likely the loss of water. In addition to this, an exothermic peak was observed again at 255°C experiencing a phase transition (Excipients, 2017). It was found that sample Y (Eudragit) had a different melting point of 45.6°C which is very different to Gellan (Ramirez-Rigo, 2014). Other prominent features of this chart were that the peaks for the complexes had diminished by 50%, the reason for this was because the addition of Gellan had impacted this.

The ideal complex 1.75 (sample s) displayed a broad peak at 95°C signifying the loss of moisture. At 245°C an exothermic peak was noticed specifying the transition period. This phase is where crystallinity was achieved from the amorphous polymer. A model study carried out by (Karthika & Vishalakshi, 2015) stated data from an experiment involving Gellan and reported an endothermic peak at 164°C. Moreover, the author presented that an exothermic was also found at 250°C. These findings are very comparable to the results achieved from the experiment in this research. Additionally, the dips that can be observed from 72°C to 99°C portrayed dehydration, moisture being lost form the samples being analysed. However, the exothermic peaks indicated the decomposition of the polymers according to the temperature (Kaur & Kaur, 2018). Also, the chart confirmed that there were changes seen in both the blends and complexed findings, giving a strong indication of the electrostatic force acting upon the

polyelectrolytes and that the original forms of the polymers had definitely altered in some ways as described.

Figure 17 shows the data from the DSC analysis from the different complexes and pure polymers for a comparison. The results indicate minor differences in comparison to Figure 16. Similarities such as the loss of moisture content were seen from 95°C and all of the samples but eudragit followed this pattern. The DSC plot followed a similar trend to Figure 16 with some minute differences such as the intensity of the peaks were different.

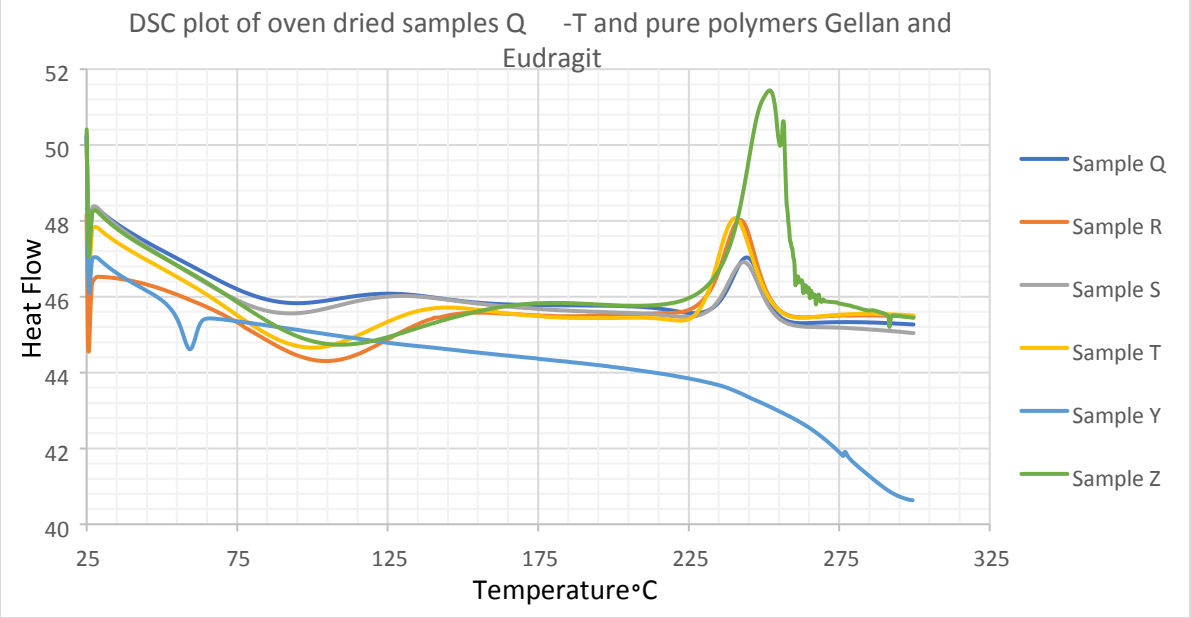

Figure 17- DSC of complexes and pure polymers

- **TGA**

Figure 18 showed the data collected from TGA analysis; the results showed that the physical blends suffered a mass loss at 225°C. However, Eudragit (sample y) began to lose mass at 275°C due to melting. Additionally, it was observed that the physical blends did not undergo a prominent amount of dehydration at the start but eventually did when moisture was lost hence, the dips after 75°C. A reason for this was because they were analysed as dry powders but they still had residual moisture content being hydrophilic polymers.

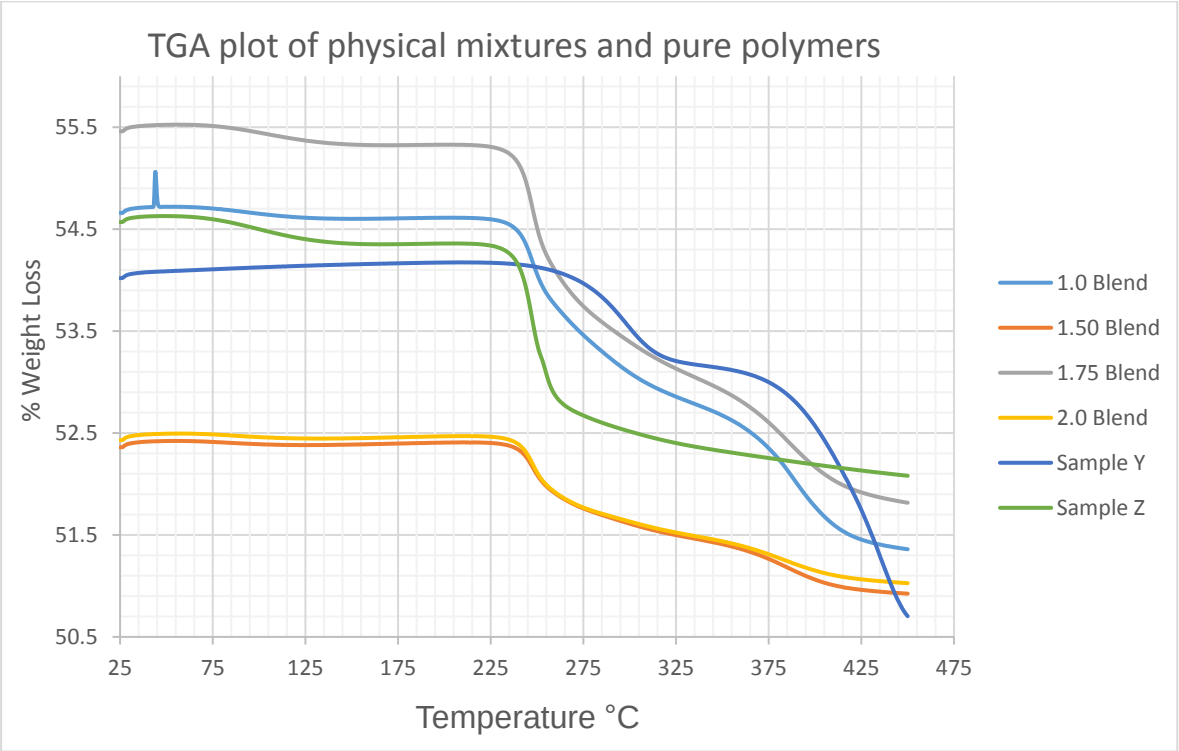

Figure 18- TGA blends and pure polymers

Figure 19 displayed the results achieved from the TGA analysis of the blank complexes and the pure polymers. This was done to determine how much weight was lost from the complexes and polyelectrolytes. The chart indicated that all of the samples undergoing analysis started to decrease in mass at 225°C but only sample Z (Gellan) showed a loss in mass at 80°C. This had occurred because Gellan goes through a gel transition phase at 80°C. Furthermore, the complexed samples (samples Q-T) shadowed a similar trend at 80°C also but showed a variance in losing mass. Sample y (Eudragit) exhibited mass changes at 275°C, this temperature was a lot higher in comparison to the other powders being tested. At 150°C specific changes were noticed because of water loss taking place (Zhu et. al. 2014). After the dehydration period secondary losses were noticed but this was now due to the detachment of the crosslinks inside the polymers.

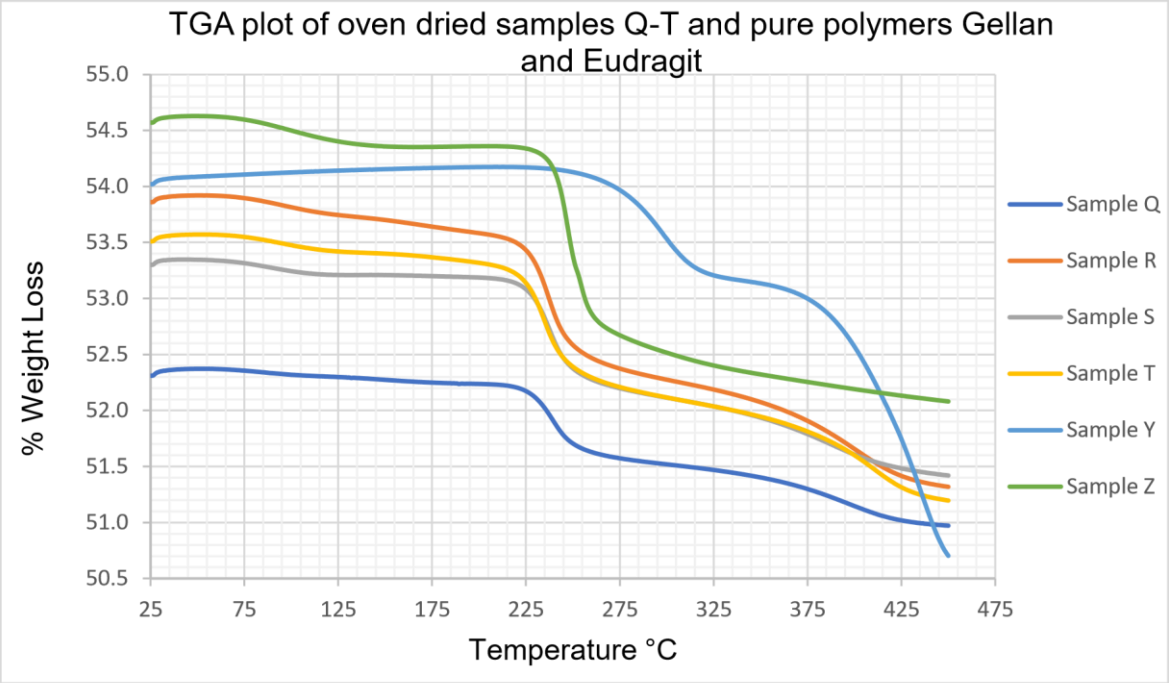

Figure 19- TGA of complexes and pure polymers

- **XRD**

Figure 20 displayed the PXRD data patterns obtained from the analysis of the blank complexed powders and pure polymers. These powders were analysed for their amorphous or crystalline properties using the Bruker Diffractometer equipment. Furthermore, the data patterns suggested that all of the samples being analysed consisted of amorphous properties. Gellan (sample z) presented a flat and broad peak in comparison to Eudragit (sample y) which was much sharper suggesting more prominent amorphous properties. The polyelectrolyte complexes also presented wide and flat patterns in comparison to sample y. A study conducted by (Quinteros et al. 2008) quantified that Eudragit alongside complexes produced from it was a polyelectrolyte completely amorphous in nature and no form of crystallinity was detected. Amorphous characteristics can affect the thermal conductivity of polymers as the size could be reduced or changed. If the polymer is amorphous the size or the particle could range from 10 nanometres to above 100 nanometres. This would mean that there would not be a stable size which could impact the final formulation and delivery (Feng et al. 2020).

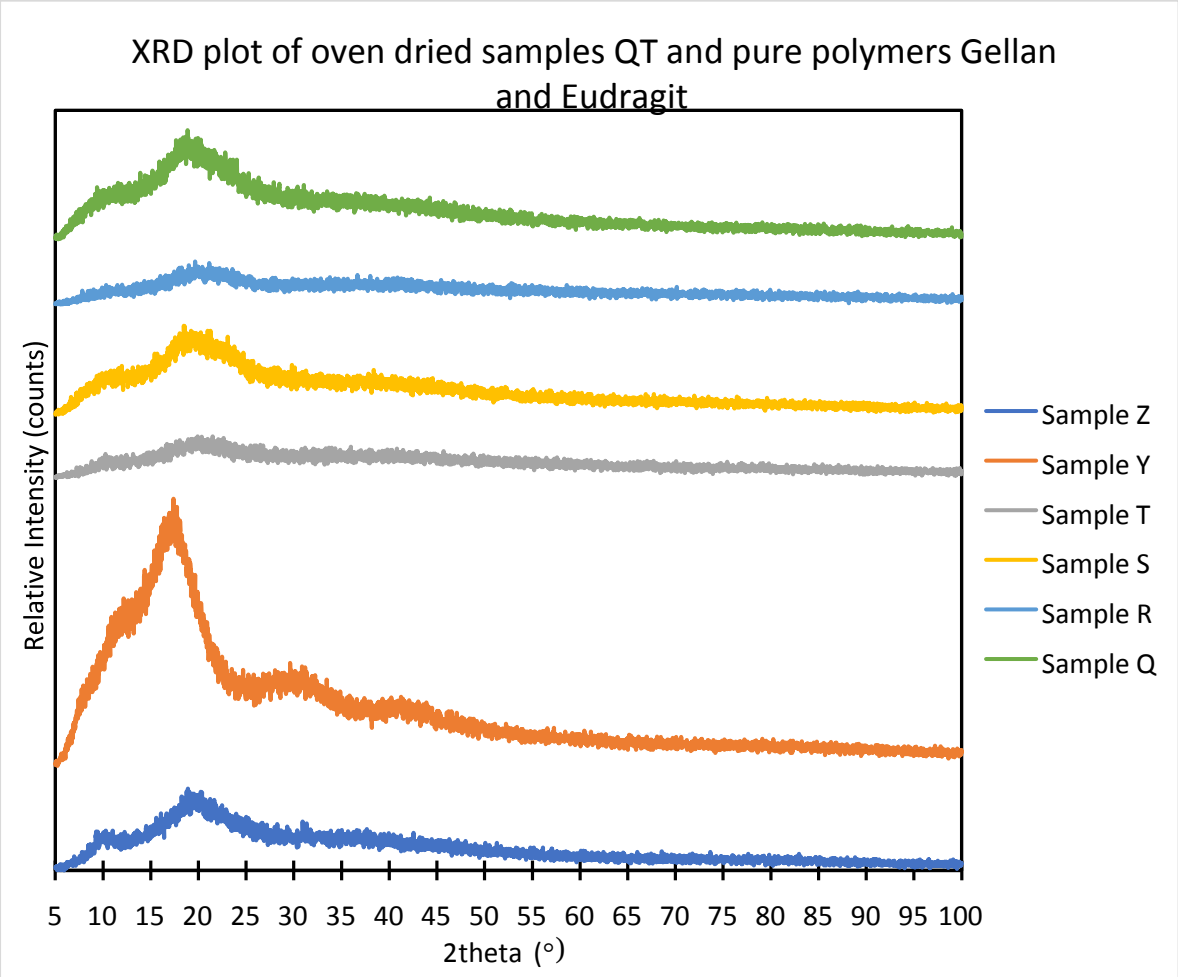

Figure 20- XRD of pure polymers and PECs

3.1.1. SEM

The scanning electron microscope images displayed in depth images of the pure polymers gellan and eudragit but also images of the new formulations at various concentrations. Figures 21-26 show the different images of the complexed polymers and also the pure polymers.

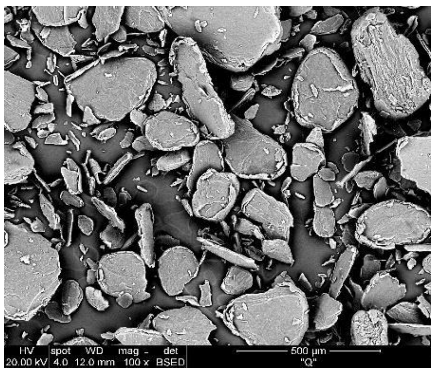

Figure 16- Sample Q (1.0)

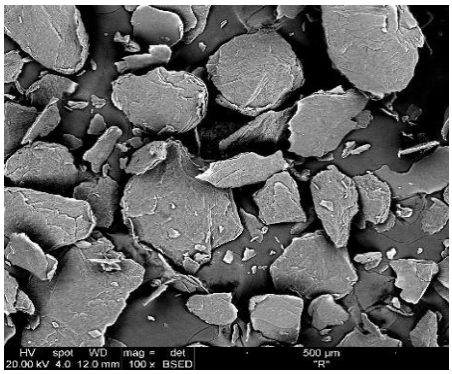

Figure 17- Sample R (1.5)

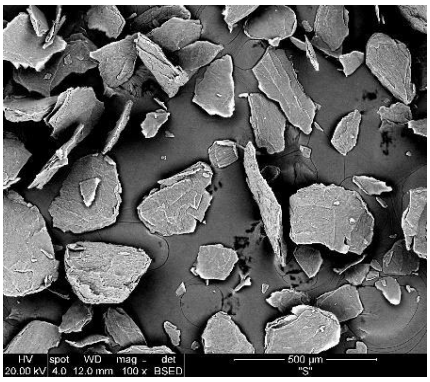

Figure 18- Sample S (1.75)

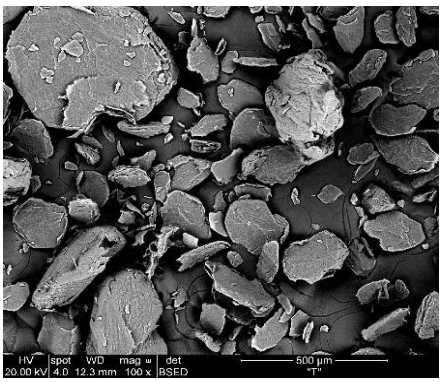

Figure 24- Sample T (2.0)

Figure 25- Sample Y Eudragit

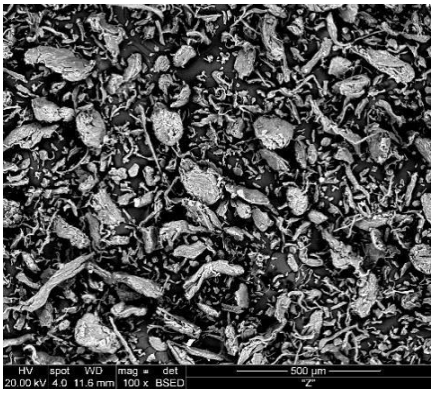

Figure 26- Sample Z Gellan

Figures 21-21 illustrate images of the weight ratios 1.0, 1.5, 1.75 and 2.0 complexed polymers GG:EE. They all appear to be irregular in shape but similar in texture. The texture was rough with thin elongated filament like detail, this could be due to the different concentrations of the polymers upon complexation and or when they had produced hard and brittle polymer films.

Figure 25 (eudragit) granules were observed under the microscope and were noticed to be very small in size but the size range did vary with some irregular shapes furthermore, eudragit appeared to have a compact structure (Oliveira et al. 2017). In addition to this, Figure 26 (gellan) did not appear to be as dense as eudragit and also was observed as irregular particles. This could be due to gellan having gel like properties but also because the gellan had to be milled down to a similar size like eudragit for uniform mixing as gellan had a larger particle size.

Chapter 4: Applications – Beads

Introduction

This chapter consists of how the ionotropic gelation method used for preparing beads was altered into producing mini discs both blank and drug entrapped with some polymer coating at different concentrations. These mini discs were prepared with both drugs ibuprofen and Propranolol while they were in their solution form.

Chapter Aim

The aim of this chapter was to achieve beads, in this case mini discs with drug entrapped in them and to then determine their efficiency on how the drug release would be affected and to see whether a sustained release formulation was achieved or not. In addition to this, the coating layer was also observed at different concentrations.

Beads method altered into mini discs

- **Ibuprofen**

Figure 27 displays the beads method that was employed and transformed into mini discs containing the active drug Ibuprofen.

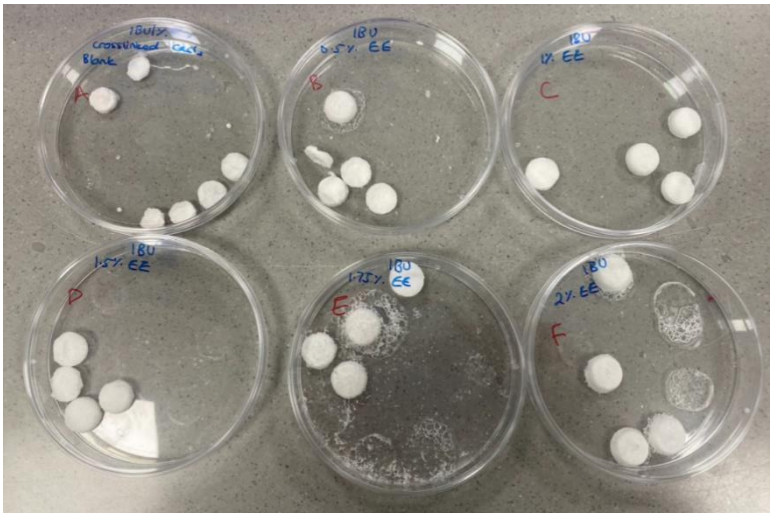

Figure 27- Discs with IBU

The mini discs were prepared and coated in numerous ways such as **A**- disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$, **B**- disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$ and coated with 0.5% Eudragit E100 solution, **C**- disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$ and coated with 1% Eudragit E100 solution, **D**- disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$ and coated with 1.5% Eudragit E100 solution, **E-** disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$ and coated with 1.75% Eudragit E100 solution, **F-** disc entrapped with 1% Ibuprofen and crosslinked with $CaCl_2$ and coated with 2.0% Eudragit E100 solution.

- **Propranolol**

Figure 28- Discs with PPN

The mini discs were prepared and coated in numerous ways such as **A**- disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$, **B**- disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$ and coated with 0.5% Eudragit E100 solution, **C**- disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$ and coated with 1% Eudragit E100 solution, **D**- disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$ and coated with 1.5% Eudragit E100 solution, **E-** disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$ and coated with 1.75% Eudragit E100 solution, **F-** disc entrapped with 1% Propranolol and crosslinked with $CaCl_2$ and coated with 2.0% Eudragit E100 solution.

Results

- **SEM/Morphology**
- **Bead size**

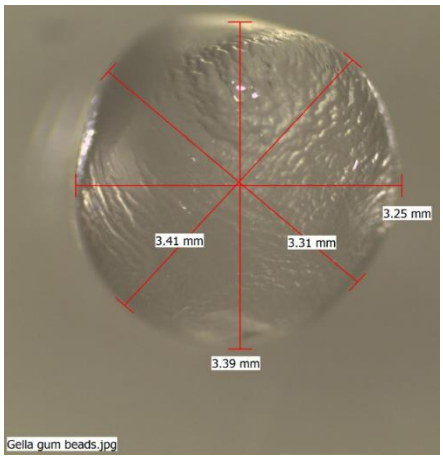

Figure 29 GG beads blank

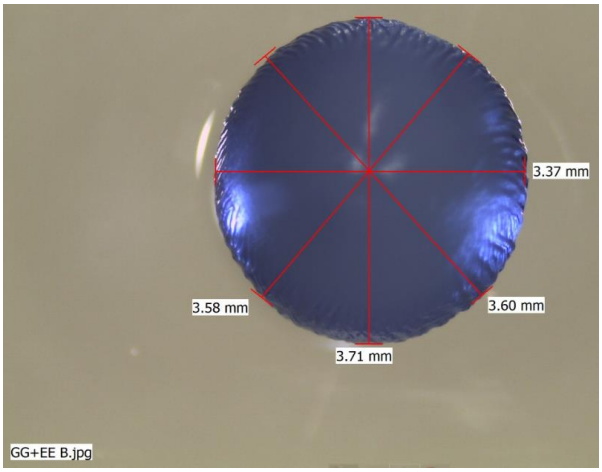

Figure 30 GG+EE bead

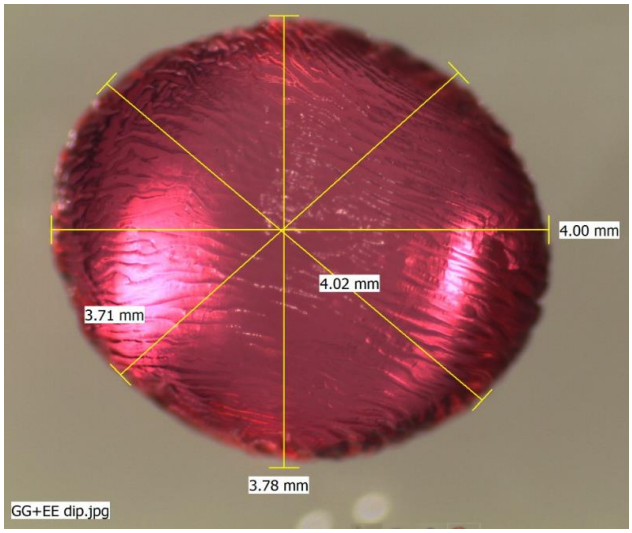

Figure 31 GG bead coated with EE

- **Drug loading /entrapment efficiency**

The mini discs were entrapped with both drugs and were then freeze dried, these were then crushed and put into volumes of buffer or acid, Readings were taken 24 hours later however, this was unsuccessful because the absorbance values were too high (refer to future work).

The results achieved were promising as the formulation developed did successfully have a coating layer of Eudragit. This was confirmed by using food colouring and immersing the discs

into various colours for confirmation. Drug entrapped discs were also successfully developed however further work needs to be carried out on the efficiency for example, dissolution studies, drug entrapment etc.

Chapter 5: Applications – Tablets

Chapter Introduction

This chapter consists of tablet compacts that were prepared with a hydraulic press. Tablet compacts were made with just the polymer to use as a matrix and then they were made with both active drugs Ibuprofen and Propranolol entrapped within during complexation and also were made without entrapping during complexation.

Chapter Aim

The aim was to determine whether a sustained release formulation could be achieved by comparing the drug release profiles of tablets made as blends including the drug and tablets made with drugs entrapped during the complexation process.

Propranolol tablets were prepared according to their drug entrapment/loading efficiency, the dissolution profile experiment was carried out in 0.1M HCl for 2 hours. Figure 32 shows the data obtained from the tablet undergoing in vitro dissolution in acid. Propranolol being a highly water-soluble drug displayed a complete 100% release profile especially in an acidic environment within 120 minutes (Ford et.al. 1985). The results showed that drug entrapped propranolol had a drug release rate of 79.4% which is not completely 100% like other findings suggested. This meant that the drug release was sustained in a way and released gradually over the 2-hour period.

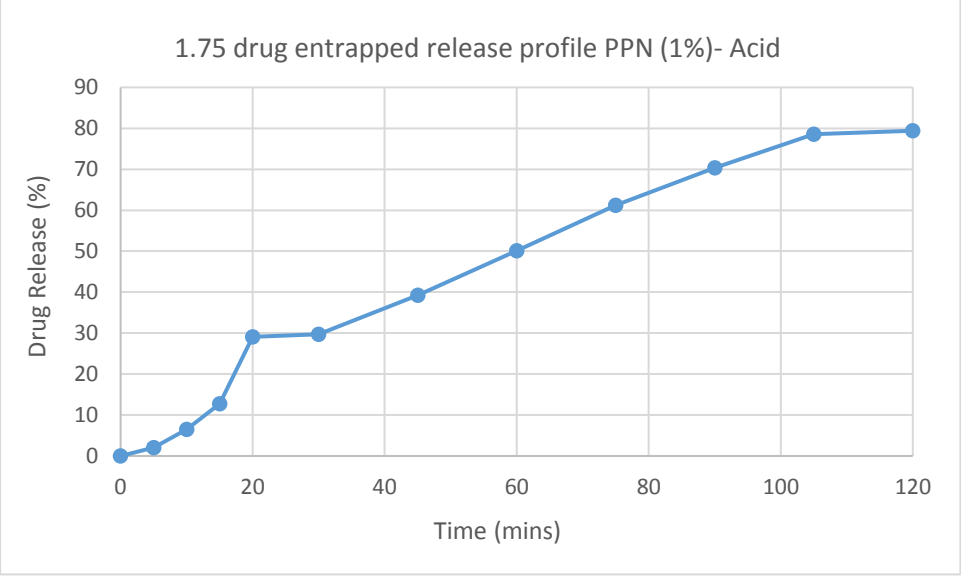

Figure 32- PPN drug release in acid

5.1.1.1.1.1.1.1. Tablets made from Polyelectrolytes without drug during complexation

Figure 33 showed the results obtained from the drug release profile of a physical blend formulation with the inclusion of the active drug propranolol. The results showed that 71% of the drug had released over the two-hour period. However, these results were the opposite of what was expected because the drug entrapped within the complexes would be expected to take longer to release through the tablet matrix in comparison to a readily uniform produced tablet which would allow a media to travel through its pores much faster.

would allow a media to travel through its pores much faster.

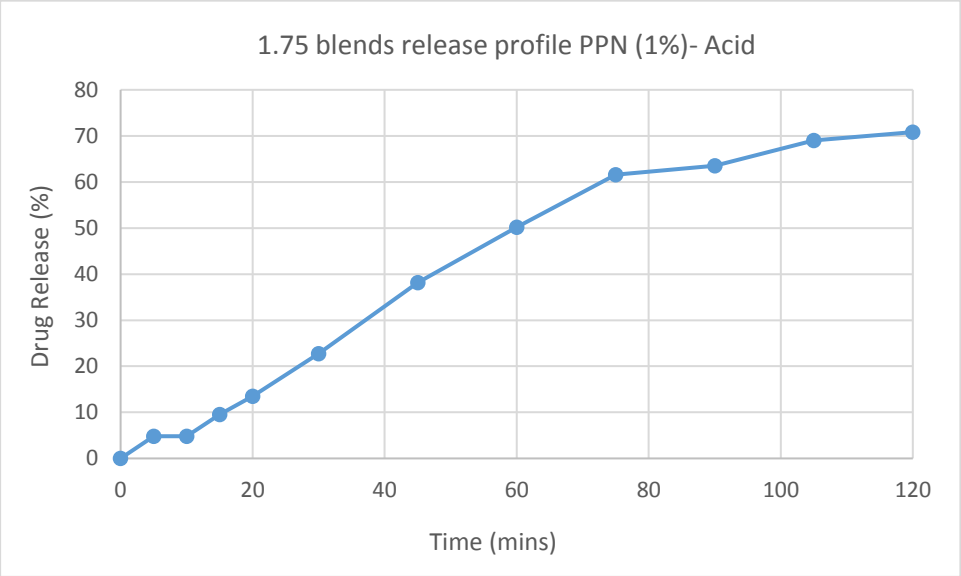

Figure 33- PPN drug release in acid

5.1.1.1.1.1.1.2. Tablets made from Polyelectrolytes with drug during complexation

Figure 34 displayed the dissolution profile of the drug entrapped propranolol tablets in buffer and achieved a drug release rate of 70.6% which is a good amount of drug release at a steady rate over the course of the experiment of 8 hours. According to (Ford et. al. 1985) the active and drug propranolol had released completely within buffer media in just 45 minutes. This meant that it was a very soluble drug and had characteristics of rapid dissolution however, this is not ideal for a sustained release profile trying to be achieved in this experiment. So, it can

be concluded that the polymers Gellan and Eudragit work well together as a matrix for a sustained release profile.

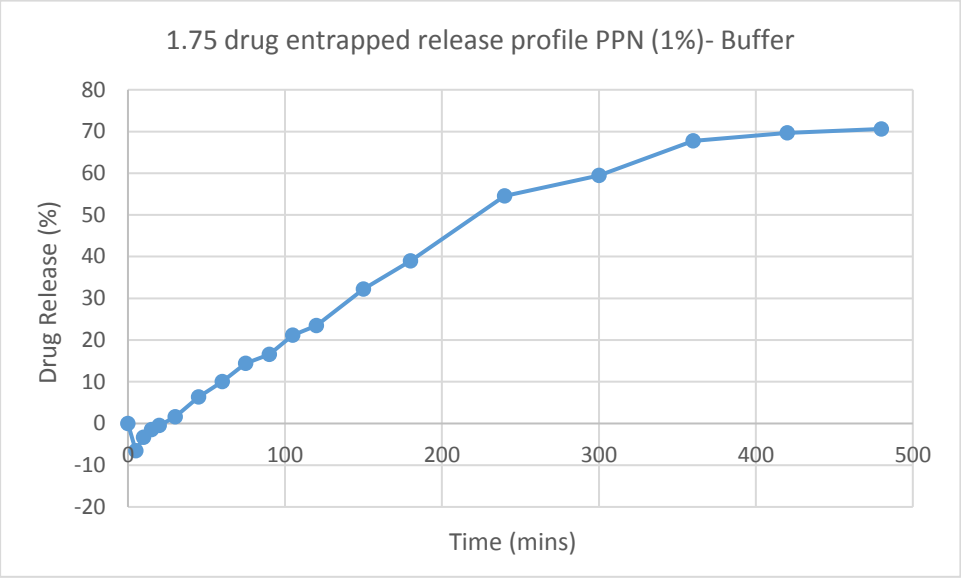

Figure 34-PPN drug release in buffer

5.1.1.1.1.1.1.3. Tablets made from Polyelectrolytes without drug during complexation

Figure 35 showed the drug release profile of the weight ratio 1.75 as a tablet matrix mixed with propranolol as a physical blend formulation. The results showed that 76% of the drug had released over the course of the 8 hours experiment. This drug release rate is higher than the drug entrapped tablets which was the desired result meaning that the drug entrapped complex tablets were effective and proved positive results.

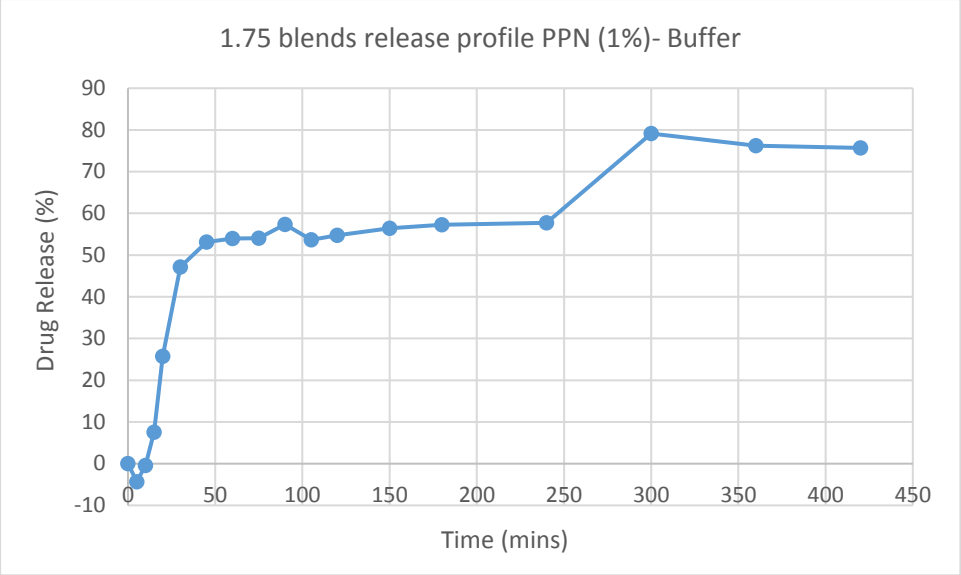

Figure 35- PPN drug release in buffer

Chapter 6: Conclusion

Overall, it was concluded that the extensive study of polyelectrolytes Gellan Gum and Eudragit E100 were successful. The polyelectrolyte complexation reaction between the two polymers was proved and the ideal weight ratio (1.75) was also determined and characterised through methods such as viscosity, zeta potential and turbidity. This weight ratio was found to be the one with promising sustained release properties. Further analytical tests were carried out such as FT-IR, SEM, TGA, DSC, XRD, Zetasizer and viscosity. These studies showed that polyelectrolyte complexation successfully happened between gellan and Eudragit because the infrared data confirmed the change in peaks. Also, the other analytical based techniques suggested the aims were met because the results indicated an overall difference to the pure polymers in comparison to the complexed polymers. Additionally, tablets incorporated with the model drugs ibuprofen and propranolol were prepared and these were tested for their drug release profile. The results showed that the drug entrapped tablets had a slower drug release rate in comparison the physical blend tablets. Similarly, beads were also prepared by the ionotropic gelation method and drug entrapment was also carried out. These beads were coated in Eudragit polymer solution at different strength. It was found that polyelectrolyte complexation was an efficacious technique employed to commence further research.

Limitations and further work

Limitations with this research were that Gellan gum is not an easy polymer to work with even though it is a versatile polyelectrolyte also, Gellan has to be used at lower concentrations for example 0.5% was workable but anything above this concentration formed a solid gel instantaneously. For example, when 1% was used it had to be used very rapidly as it solidified within seconds. Moreover, Ibuprofen was a poor drug to work with because of its poor solubility it did not provide the results that were planned for. In the future, a different drug from the same family such as flurbiprofen, diclofenac or aspirin would be better to use to achieve the results that were initially planned for.

To reduce human error which was possibly part of the dissolution studies in this project an automatic dissolution apparatus would have been preferred, not only to use time wisely but also to have more accurate and precise data because the manual readings were not taken at exact times because taking a larger number of samples at one time is not possible. In addition to this, some of the data was not fully obtained because the TGA and DSC apparatus were not available.

Because of the sudden shutdown of the university due to the ongoing global pandemic some of the intended research had to be abandoned which includes tablet testing including hardness, friability, tablet thickness and tablet diameter. Furthermore, dissolution studies were badly effected due to only having a manual dissolution apparatus available which led to a delay in experiments which could have been done quicker.

References

- Lankalapalli, S., & Kolapalli, V. R. M. (2009). PECs: A Review of their Applicability in Drug Delivery Technology. *Indian Journal of Pharmaceutical Sciences*, 71(5), 481–487. http://doi.org/10.4103/0250-474X.58165

- Giavasis et al., 2000 I. Giavasis, L.M. Harvey, B. McNeil GG

  Critical Reviews in Biotechnology, 20 (3) (2000), pp. 177-211

- Smith et al., 2007 A.M. Smith, R.M. Shelton, Y. Perrie, J.J. Harris An initial evaluation of GG as a material for tissue engineering applications Journal of Biomaterials Applications, 22 (3) (2007), pp. 241-254

- *GG* (2014). (4th ed.) Oxford University Press.

- Osmałek, T., Froelich, A., & Tasarek, S. (2014). Application of GG in pharmacy and medicine. *International Journal of Pharmaceutics, 466*(1-2), 328-340. doi:10.1016/j.ijpharm.2014.03.038

- Miyazaki, S., Aoyama, H., Kawasaki, N., Kubo, W., & Attwood, D. (1999). In situ-gelling gellan formulations as vehicles for oral drug delivery. *Journal of Controlled Release, 60*(2-3), 287-295. doi:10.1016/S0168-3659(99)00084-X

- Norton et al., 1984 I.T. Norton, D.M. Goodall, S.A. Frangou, E.R. Morris, D.A. Rees Mechanism and dynamics of conformational ordering in xanthan polysaccharides J. Mol. Biol., 175 (1984), pp. 371-394

- Mahdi, M. H., Conway, B. R., & Smith, A. M. (2015). Development of mucoadhesive sprayable GG fluid gels. *International Journal of Pharmaceutics, 488*(1-2), 12-19. doi:10.1016/j.ijpharm.2015.04.011

- Moustafine, R. I., Kabanova, T. V., Kemenova, V. A., & Van den Mooter, G. (2005). Characteristics of interPECs of EE with eudragit L100. *Journal of Controlled Release, 103*(1), 191-198. doi:10.1016/j.jconrel.2004.11.031

- S.L. Dakhara, C.C. Anajwala Polyelectrolyte complex: a pharmaceutical review Syst. Rev. Pharm., 1 (2010), p. 127 Google Scholar

- Yilmaz, T., Maldonado, L., Turasan, H., & Kokini, J. (2019). Thermodynamic mechanism of particulation of sodium alginate and chitosan PECs as a function of charge ratio and order of addition. *Journal of Food*

- *Engineering, 254*, 42-50. doi:10.1016/j.jfoodeng.2019.03.002

- H.G. Bungenberg de Jong, H.R. Kruyt Coacervation (partial miscibility in colloid systems) Proceedings Koninklijke Nederlandse Akademie Van Wetenschappen, 32 (1929), pp. 849-856 View Record in ScopusGoogle Scholar

- V.J. Vandenberg, *et al.*The synthesis and solution properties of some rigid-chain, water-soluble polymers: poly[*NN*′-(sulfophenylene)phthalamide]s and poly[*NN*′-(sulfo-pphenylene)pyromellitimide

- J. Polym. Sci. A Polym. Chem., 27 (1989), pp. 3745-3757 CrossRefView Record in ScopusGoogle Scholar
- H. Dautzenberg, *et al.*Polyelectrolytes: formation, characterization and application Polym. Int., 38 (1994), p. 106 View Record in ScopusGoogle Scholar
- Morris, E. R., Nishinari, K., & Rinaudo, M. (2012). Gelation of gellan – A review. *Food Hydrocolloids*, 28(2), 373-411. doi:10.1016/j.foodhyd.2012.01.004
- D.K. Abhijeet, *et al.*PECs: mechanisms, critical experimental aspects, and application Artif. Cells Nanomed. Biotechnol., 44 (2016), pp. 1615-1625Google Scholar
- M. Muller, *et al.*Polyelctrolyte complex nanoparticles of poly(ethyleneimine)and poly(acrylic acid): preparation and applications
- Polymers, 3 (2011), pp. 762-778
- Tan, C., Xie, J., Zhang, X., Cai, J., & Xia, S. (2016). Polysaccharide-based nanoparticles by chitosan and gum arabic polyelectrolyte complexation as carriers for curcumin. *Food Hydrocolloids, 57*, 236-245. doi:10.1016/j.foodhyd.2016.01.021
- Li, J., Li, L., Wang, L., Wang, Y., Jiang, S., Zhang, X., . . . Mao, S. (2014). Insights into the mechanisms of chitosan–anionic polymers-based matrix tablets for extended drug release.*International Journal of Pharmaceutics, 476*(1-2), 253-265. doi:10.1016/j.ijpharm.2014.09.057
- Mahdi, M. H., Conway, B. R., Mills, T., & Smith, A. M. (2016). GG fluid gels for topical administration of diclofenac. *International Journal of Pharmaceutics, 515*(1-2), 535-542. doi:10.1016/j.ijpharm.2016.10.048
- Samal, S. K., Dash, M., van Vlierberghe, S., Kaplan, D., Chiellini, E., van Blitterswijk, C., . . . Dubruel, P. (2012). Cationic polymers and their therapeutic potential. *Chemical Society Reviews, 41*(21), 7147-7194. doi:10.1039/c2cs35094g
- Bourganis, V., Karamanidou, T., Kammona, O., & Kiparissides, C. (2017). PECs as prospective carriers for the oral delivery of protein therapeutics. *European Journal of Pharmaceutics and Biopharmaceutics, 111*, 44-60. doi:10.1016/j.ejpb.2016.11.005
- Beakawi Al-Hashemi, H. M., & Baghabra Al-Amoudi, O. S. (2018). A review on the angle of repose of granular materials. *Powder Technology, 330*, 397-417. doi:10.1016/j.powtec.2018.02.003

- Alhaique, F., Santucci, E., Carafa, M., Coviello, T., Murtas, E., & Riccieri, F. M. (1996). Gellan in sustained release formulations: Preparation of gel capsules and release studies.*Biomaterials, 17*(20), 1981-1986. doi:10.1016/0142-9612(96)00017-8

- Lankalapalli, S., & Kolapalli, R.M. (2012). Biopharmaceutical evalusation of diclofenac sodium controlled release tablets prepared from gum karaya – chitosan PECs.Drug development and Industrial Pharmacy, 38(7), 815-824. Doi:10.3109/03639045.2011.630006

- Dakhara, S., & Anajwala, C. (2010). Polyelectrolyte complex: A pharmaceutical review. *Systematic Reviews in Pharmacy*, *1*(2), 121.

- ApurvaSrivastava, Tejaswita Yadav, Soumya Sharma, Anjali Nayak, Akanksha Akanksha Kumari, Nidhi Mishra* (2016) Polymers in Drug Delivery DOI: 10.4236/jbm.2016.41009

- Kaur, J., & Kaur, G. (2018). Optimization of pH conditions and characterization of polyelectrolyte complexes between gellan gum and cationic guar gum. *Polymers for Advanced Technologies, 29*(12), 3035-3048. doi:10.1002/pat.4424

- Mallick, S., Kumar Pradhan, S., Chandran, M., Acharya, M., Digdarsini, T., & Mohapatra, R. (2011). Study of particle rearrangement, compression behavior and dissolution properties after melt dispersion of ibuprofen, avicel and aerosil. Results in Pharma Sciences, 1(1), 1-10. doi:10.1016/j.rinphs.2011.05.003

- Chakraborty, S., Jana, S., Gandhi, A., Sen, K. K., Zhiang, W., & Kokare, C. (2014). Gellan gum microspheres containing a novel α-amylase from marine nocardiopsis sp. strain B2 for immobilization. International Journal of Biological Macromolecules, 70, 292-299. doi:10.1016/j.ijbiomac.2014.06.046

- Zia, K. M., Tabasum, S., Khan, M. F., Akram, N., Akhter, N., Noreen, A., & Zuber, M. (2018). Recent trends on gellan gum blends with natural and synthetic polymers: A review. *International Journal of Biological Macromolecules, 109*, 1068-1087. doi:10.1016/j.ijbiomac.2017.11.099

- Schanze, K. S., & Shelton, A. H. (2009). Functional polyelectrolytes. *Langmuir, 25*(24), 13698-13702. doi:10.1021/la903785g

- Zhang, B., Tao, H., Niu, X., Li, S., & Chen, H. (2017). Lysozyme distribution, structural identification, and in vitro release of starch-based microgel-lysozyme complexes. *Food Chemistry, 227*, 137-141. doi:10.1016/j.foodchem.2017.01.073

- Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Filipic, M., Frutos, M. J., . . . EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). (2018). Re-evaluation of gellan gum (E 418) as food additive. *EFSA Journal, 16*(6), n/a. doi:10.2903/j.efsa.2018.5296

- Facchi, D. P., Lima, A. C., de Oliveira, J. H., Lazarin-Bidóia, D., Nakamura, C. V., Canesin, E. A., . . . Martins, A. F. (2017). Polyelectrolyte complexes based on alginate/tanfloc: Optimization, characterization and medical application. *International Journal of Biological Macromolecules, 103*, 129-138. doi:10.1016/j.ijbiomac.2017.05.03

- Mahajan, A., & Aggarwal, G. (2011). Smart polymers: innovations in novel drug delivery. *Int. J. Drug Dev. Res*, *3*(3), 16-30.

- Mariod, A. A., & Fadul, H. (2013). Gelatin, source, extraction and industrial applications. *Acta Scientiarum Polonorum Technologia Alimentaria*, *12*(2), 135-147.

- Anitha A, Deepagan VG, Divya Rani VV, Menon D, Nair SV, Jayakumar R. 2011. Preparation, characterization, in vitro drug release and biological studies of curcumin loaded dextran sulphate–chitosan nanoparticles. Carbohydr Polym. 84:1158–1164.

- Martins AF, Bueno PVA, Almeida EAMS, Rodrigues FHA, Rubira AF, Muniz EC. 2013. Char acterization of N-trimethyl chitosan/alginate complexes and curcumin release. Int J Biol Macromol. 57:174–184.

- Polexe RC, Terrat C, Verrier B, Cuvilier A, Champier G, Delair T. 2013. Elaboration of targeted nanodelivery systems based on colloidal polyelectrolyte complexes (PEC) of chitosan (CH)-dextran sulphate (DS). Eur J Nanomed. 5:39–49.

- J. Milan and G.Maleki, in “Food Industrial Processes – Methods and Equipment”, edited by B. Valdez, InTech Europe, Croatia, 2012, pp. 17-38.

- M. Popa, C. L. Dumitriu and S. Vasiliu, Cellulose Chem. Technol., 38, 363 (2004).

- T. Feng, J. He, A. Rai, D. Hun, J. Liu, S. Shrestha- size effects in the thermal conductivity of amorphous polymers, Applied 14, 044023- Published 14 October 2020